Assessment of the mastitis situation in Canada

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Abstract

The reason for initiation of the studies described in this thesis is that the Canadian Bovine Mastitis Research Network needed to acquire knowledge of the distribution of mastitis pathogens across Canada to before starting projects to improve the udder health status of the national dairy herd. The aims of this thesis were, therefore, to estimate: 1) the incidence rate of clinical mastitis (IRCM) and pathogen-specific IRCM per region on Canadian dairy farms and the association of pathogen-specific IRCM with bulk milk somatic cell count (BMSCC) and barn type; 2) associations of risk factors with overall and pathogen-specific IRCM on Canadian dairy farms; 3) the adoption proportion of recommended mastitis preventive management practices on Canadian dairy farms; 4) the herd-level prevalence of contagious mastitis pathogens; and 5) associations of certain management practices with the isolation of contagious mastitis pathogens from bulk tank milk. Overall mean IRCM was 22 cases per 100 cow-years in the selected herds. There was no association between BMSCC and overall IRCM, but *Escherichia coli* and culture-negative IRCM was highest in low and medium BMSCC herds. Herds in Ontario and Québec had the highest IRCM, and herds in the Western provinces had the lowest IRCM. The most frequently isolated pathogens from clinical mastitis in Canada were *Staphylococcus aureus*, *E. coli*, *Streptococcus uberis*, and coagulase-negative staphylococci. *Escherichia coli* IRCM was relatively higher in Ontario than in other regions, but *Streptococcus dysgalactiae* IRCM was highest in Québec. *Staphylococcus aureus* is present in bulk tank milk of nearly all Canadian dairy farms, whereas *Streptococcus agalactiae* may be near extinction in Canada. Adoption of most of these recommended mastitis management practices is high in Canadian dairy herds. We demonstrated that season had an effect on all udder health parameters, BMSCC, individual cow somatic cell count (ICSCC), and IRCM. And finally, that quarter SCC fluctuates during and between milking which has consequences for implementing udder health programs that use ICSCC to identify cows with an intramammary infection. The Canadian mastitis control program should not only focus on reducing *Staph. aureus* and information transfer, but should also find ways to motivate producers to implement these practices.

This thesis is dedicated to

Opa Schal Tinus, my grandfather (1909 – 2003)

- For his inspiration -

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LIST OF ABBREVIATIONS

BMSCC	Bulk Milk Somatic Cell Count
CBMRN	Canadian Bovine Mastitis Research Network
CFU	Colony Forming Units
CMT	California Mastitis Test
CNS	Coagulase-negative Staphylococci
DCT	Dry Cow Treatment
DHI	Dairy Herd Improvement
DIM	Days In Milk
ICSCC	Individual Cow Somatic Cell Count
IMI	Intramammary Infection
IRCM	Incidence Rate of Clinical Mastitis
PEI	Prince Edward Island
POST-AM	Immediately after AM milking
PRE-AM	Immediately before AM milking
PRE-PM	Immediately before PM milking
SCC	Somatic Cell Count

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Mastitis

Despite considerable research effort, mastitis remains the most costly disease on a dairy farm, not only due to suboptimal milk production, but also due to discarded milk, treatment costs, early culling and death, veterinary fees, and labor costs (Schepers and Dijkhuizen, 1991). Discarded milk and lowered milk production account for approximately 80% of costs associated with mastitis (Reneau and Packard, 1991). Lower milk quality because of increased somatic cell count (SCC) in the milk decreases shelf life of milk and cheese making quality (Klei et al., 1998; Ma et al., 2000). Additionally, the importance of mastitis in public perception should not be overlooked. The general public is more and more concerned with animal welfare, possible antibiotic residues in the milk, and a disease such as mastitis that can cause severe distress to the cow should not be ignored (Bradley, 2002).

1.2 Mastitis pathogens

Mastitis, or inflammation of the udder, is most often caused by a bacterial infection. Watts (1988) identified 137 species of microorganisms that can cause mastitis, but in clinical cases of mastitis, staphylococci, streptococci, and coliform organisms are isolated most often. These pathogens are, based on their primary reservoir, usually categorized as contagious (*Staphylococcus aureus* and *Streptococcus agalactiae*), environmental (*Escherichia coli* and *Streptococcus uberis*), or skin flora opportunists (coagulase-negative staphylococci (CNS)). However, there is now an

increasing body of evidence that this classification may not be as clear or mutually exclusive as previously thought (Bradley, 2002; Zadoks et al., 2003).

The most important contagious mastitis pathogens are *Staph. aureus*, *Strep. agalactiae*, and *Mycoplasma* spp. *Streptococcus dysgalactiae* can also be considered a contagious mastitis pathogen (Fox and Gay, 1993). The contagious mastitis pathogens reside primarily in the cow's udder and are most often transmitted from cow to cow during milking (Fox and Gay, 1993). *Staphylococcus aureus* is a gram-positive bacterium, can cause subclinical and clinical mastitis in dairy cows, and is one of the most important causes of intramammary infections (IMI) in the dairy herd (Barkema et al., 1997). The pathogen spreads easily within dairy herds, causing chronic mastitis that is most often subclinical (Fox and Gay, 1993). *Streptococcus agalactiae* is a gram-positive bacterium, is a contagious obligate parasite of the bovine mammary gland, and most often causes subclinical mastitis and elevated SCC (Pyörälä, 1995; Keefe et al., 1997). It generally causes a low-grade persistent type of infection and does not have a high self-cure rate. *Mycoplasma* are pleomorphic bacteria that lack a cell wall, are contagious, and can cause high SCC and chronic clinical mastitis (Bushnell, 1984). With respect to mastitis in dairy cows, *Mycoplasma* spp. are highly contagious and economically important causes of milk loss and increased culling in infected cows (Gonzalez et al., 1992). The most prevalent and economically most important *Mycoplasma* species is *M. bovis* (Fox et al., 2005).

The most frequently isolated environmental pathogens are *E. coli*, *Strep. uberis*, and *Klebsiella* spp. *Escherichia coli* is the pathogen most frequently isolated from clinical mastitis cases worldwide. Particularly in herds with low bulk milk SCC (BMSCC), incidence of *E. coli* can be high (Barkema et al., 1998). *Klebsiella* is an

emerging mastitis pathogen in the U.S. (Zadoks and Munoz, 2007). Both *E. coli* and *Klebsiella* spp. are gram-negative organisms. Approximately 80 to 90% of gram-negative IMI result in clinical mastitis (Smith et al., 1985). *Streptococcus uberis* is a widely occurring causative agent of mastitis in modern dairy herds. This pathogen is responsible for the majority of clinical and subclinical mastitis cases in New Zealand and the UK, and ranks among the most prevalent causes of mastitis in the U.S.A. and the Netherlands (Zadoks et al., 2001). There has been little reduction in the incidence of *Strep. uberis* mastitis over the past 30 years in the U.K. (Leigh, 1999). Because in North America the incidence of non-*agalactiae* streptococci are reported as a group, no data on the pathogen-specific incidence of *Strep. uberis* mastitis are available on this continent (Smith et al., 1985; Sargeant et al., 1998b).

1.3 Clinical mastitis

Clinical mastitis can be defined as a 'farmer observed abnormality of the milk and/or the udder' (Schukken and Kremer, 1996). Clinical mastitis then, is an observable disease. Cows are visibly sick, or the milk is visibly abnormal. Clinical mastitis continues to be a significant problem on dairy farms (Barkema et al., 1998; Sargeant et al., 1998b). The incidence rate of clinical mastitis in herds with low BMSCC is sometimes very high, mainly due to infections with environmental pathogens, such as *E. coli* (Barkema et al., 1998). The significance of environmental pathogens cannot be determined using bulk milk because contamination from the environment is unavoidable (Jayarao and Wolfgang, 2003). For this reason sampling of clinical mastitis cases is necessary.

Several studies have been conducted to estimate the incidence rate of clinical mastitis (IRCM) in Europe (Schukken et al., 1989b; Barkema et al., 1998; Peeler et al., 2000; Barnouin et al., 2005; O'Reilly et al., 2006; Nyman et al., 2006; Bradley et al., 2007), North America (Dohoo et al., 1983; Erskine et al., 1988; Bartlett et al., 1992; Sargeant et al., 1998b), Australia (Daniel et al., 1982), New Zealand (McDougall, 1999), and Africa (Kivaria et al., 2006). Distribution of pathogens isolated from clinical mastitis samples differs considerably among the countries and even studies within a country. In Norway, for example, *Staphylococcus aureus* is the most frequently isolated bacteria in clinical mastitis samples followed by *Streptococcus dysgalactiae* (Reksen et al., 2006). In Midwest USA, in low BMSCC herds, coliforms were the most frequently isolated bacteria (Erskine et al., 1988). In Europe, clinical *Klebsiella* mastitis occurs less frequent than clinical *Escherichia coli* mastitis, while in the US *Klebsiella* is of equal importance (Barkema et al., 1998; Roberson et al., 2004). In New Zealand, coliforms are less important as mastitis causing pathogens, and *Streptococcus uberis* is the main concern in both clinical and subclinical mastitis in all herds (McDougall, 1998).

1.4 Subclinical mastitis

Somatic cell count is the most frequently used indicator of subclinical mastitis in dairy cattle. Subclinical mastitis accounts for high economic losses in the dairy industry (Tyler et al., 1989). The most important cause of increased SCC is a bacterial infection of the mammary gland (Dohoo and Meek, 1982; Harmon, 1994). Non-bacterial factors that affect SCC include age, stage of lactation, season, stress, management, day-to-day variation, and diurnal variation. Diurnal variation of SCC could have consequences for

interpretation of SCC data if milk samples are collected at any time other than immediately before or during milking (Dohoo and Meek, 1982). Milk samples for SCC analysis are routinely collected at milking time as part of Dairy Herd Improvement programs. For researchers and veterinarians, sample collection during milking may not always be feasible, and could therefore result in misinterpretation of the results, such as a false-positive IMI status. Because SCC is an important indicator for subclinical mastitis, it is important to know how SCC behaves. Somatic cells consist mainly of polymorphonuclear leucocytes (PMNL), macrophages and monocytes, lymphocytes, squamous cells, and a fraction of degenerated cells (Miller et al., 1990). Some of these cells have a specific function in the immune system and increases in SCC could be the result of an increase of one specific cell type (Leitner et al., 2000; Paape et al., 2002).

1.5 Bulk milk

The contagious mastitis pathogens *Staphylococcus aureus, Streptococcus agalactiae*, and *Mycoplasma* spp. reside primarily in the cow's udder; therefore, when they are found in bulk milk, these mastitis causing organisms are strong indicators of the presence of IMIs in the herd (Gonzalez et al., 1986; Fox et al., 2005). Bulk tank milk culture may be used as a monitoring tool in the control and evaluation of clinical and subclinical mastitis (Jayarao and Wolfgang, 2003). This tool may be useful while investigating potential milk quality problems on a dairy farm, such as increased bacterial count or increased BMSCC are being investigated (Farnsworth, 1993; Jayarao and Wolfgang, 2003). Bulk milk culture is a cheap and convenient method of evaluating milk quality compared with the collection and culturing of individual cow milk samples,

and it may be a useful tool for estimating herd level prevalence of contagious mastitis pathogens. To retrieve the most information out of a bulk milk sample, it is necessary that the sample is fresh (Jayarao et al., 2004). Bacteria counts can be compromised if milk samples were frozen and thawed, which is the case for coliforms, but not for streptococci and *Staph. aureus* (Schukken et al., 1989a). Biddle et al. (2004) found that frozen storage and thawing of milk samples from cows with *Mycoplasma* IMI is harmful to *Mycoplasma* organisms in the milk. It is very likely that this is valid for bulk milk samples as well. However, because of the long distances within Canada, it is practically impossible to collect fresh bulk milk samples for culture in a single laboratory.

1.6 Mastitis control

Neave et al. (1969), proposed the "standard mastitis control plan," better known as the 5-point mastitis control program. Where it has been applied, it has had considerable success in reducing incidence of subclinical and clinical mastitis in dairy herds (Bradley, 2002). The 5-point mastitis control program was basically geared towards contagious mastitis pathogens, *Strep. agalactiae*, *Staphylococcus aureus,* and to a lesser extent, *Strep. dysgalactiae.* The plan focused, given the name, on 5 points in mastitis management: rapid identification and treatment of clinical cases, routine whole herd antibiotic dry cow therapy, post-milking teat disinfection, culling of chronically infected cows, and the routine maintenance of the milking equipment. However, after successfully controlling the contagious mastitis pathogens, the plan was less effective to address problems with environmental pathogens, primarily because the management practices out of the 5 point plan do not directly affect the primary reservoir of

environmental pathogens (Smith et al., 1985). It was for this reason that the National Mastitis Council (NMC) developed a new 10-step plan. This plan includes some general management advice, review of mastitis data and udder health, and adds focus on a clean, dry, and comfortable environment for the cows to the other control measures.

1.7 Risk factors for mastitis

Risk factors that are associated with the IRCM can be divided into three distinct groups based on the epidemiologic triad of host, environment, and pathogen. Host risk factors for IRCM include breed of the cow (Schukken et al., 1990; Nyman et al., 2006), high milk production (Schukken et al., 1990; Barnouin et al., 2005; O'Reilly et al., 2006), leaking of milk (Schukken et al., 1990; Peeler et al., 2000; O'Reilly et al., 2006), and decreased resistance to IMI due to teat end callosity (Neijenhuis et al., 2001) or vitamin E and Se deficiency (Erskine, 1993). Environmental risk factors include straw or wood shavings as bedding material in stalls which increases the bacterial count for *Staphylococcus aureus* and *Streptococcus uberis*, and *Klebsiella* spp., respectively (Rendos et al., 1975; Bramley et al., 1984), inadequate ventilation such as air inlet along roof which is associated with a decreased *Escherichia coli* IRCM (Schukken et al., 1991), and high temperature and humidity (Morse et al., 1988; Hogan and Smith, 1997; Hogan and Smith, 2003). The latter two risk factors are not always manageable on a dairy farm, especially not if the herd is on pasture part of the year. Seasonal influence on incidence rate of clinical mastitis (Morse et al., 1988; Hogan et al., 1989), subclinical mastitis (Green et al., 2006), and bulk milk SCC (Schukken et al., 1992) has been reported. In herds with year-round-calving, SCC had a seasonal pattern, with the highest

BMSCC occurring from July to October (Schukken et al., 1993; Sargeant et al., 1998a). Seasonal patterns can also be found in individual cow SCC, with the highest SCC in July and August (Bodoh et al., 1976; Salsberg et al., 1984). Green et al. (2006) suggested that part of the seasonal variation of BMSCC was caused by the larger proportion of cows with prolonged high SCC in the summer.

Because the epidemiology of each pathogen is unique, the effect of each pathogen on BMSCC and IRCM and its association with climatic, environmental, and management risk factors might be different.

1.8 Current situation in Canada

Canadian studies on IRCM are scarce and limited historically and geographically (Dohoo et al., 1983; Meek et al., 1986; Sargeant et al., 1998b; Van Dorp et al., 1999; McLaren et al., 2006). A study conducted in 1993-1996 in 32 herds in British Columbia found very low lactational IRCM based on farm records, ranging from 5.6 to 10.5% in first lactation and fifth and greater lactation cows, respectively (Van Dorp et al., 1999). More recent studies in 48 and 65 selected commercial Ontario dairies estimated the lactational IRCM to be 22 and 20%, respectively (Sargeant et al., 1998b; McLaren et al., 2006). In an earlier study, also in Ontario, as part of a disease cohort study, lactational IRCM was reported to be 17% (Dohoo et al., 1983). In most studies, no bacteriology of milk samples was performed, except in the study of Sargeant et al. (1998b), who isolated *Staphylococcus* spp. other than *Staph. aureus* most often from clinical mastitis samples, followed by coliforms and *Streptococcus* spp.

Several studies in the United States and Europe have estimated the herd-level prevalence of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp. (Vecht et al., 1989; Khaitsa et al., 2000; Jayarao et al., 2004; Tenhagen et al., 2006). However, only a few prevalence studies have been conducted in Canada. *Streptococcus agalactiae* prevalence in Canadian bulk milk ranged between 6% in Alberta (Schoonderwoerd et al., 1993) and 43% in Québec (Guillemette et al., 1992). In a study on Ontario dairy farms, 58 out of 59 bulk milk samples were *Staph. aureus*-positive (Kelton et al., 1999). No studies have been conducted on the prevalence of *Mycoplasma* species in Canadian dairy herds since 1972 (Ruhnke et al., 1976).

A number of studies in Canada investigated management practices on dairy farms (Spicer et al., 1994; Sargeant et al., 1997). However, these studies did not focus on mastitis management alone and were restricted temporally or geographically. Therefore, adoption of these management practices by Canadian dairy producers is unknown. The combination of knowledge of the prevalence of contagious pathogens and adoption of mastitis management practices and the association of the prevalence with these practices will be an important source to give direction to herd-level, province- and nationwide mastitis prevention programs.

1.9 Canadian Bovine Mastitis Research Network

In 2001, 38 Canadian researchers founded the Canadian Bovine Mastitis Research Network to "*mobilize national and international scientific and financial resources to decrease the incidence of mastitis, reduce financial losses, and maintain milk quality through concerted research, and effective and rapid transfer of results to*

end-users." (http://www.mastitisnetwork.org). They decided that before starting projects aimed at improving the udder health status of the national herd, first information should be collected on the distribution of pathogens in clinical and subclinical mastitis and the current state of adoption of control programs. This realization triggered the initiation of this Ph.D. project.

1.10 Specific objectives of this thesis

Because mastitis is a complex disease involving many bacteria and modes of spread, studies using both bulk tank and clinical mastitis samples are necessary to properly quantify the disease. Bulk tank samples are useful for defining herd infection with pathogens whose main reservoir in the herd is the udder (contagious bacteria). Individual cow clinical mastitis samples are required to ascertain environmental bacteria patterns because these organisms may be found in the bulk tank from non-cow sources (contamination from the environment). Individual cow samples are also required to obtain subclinical mastitis information. An elevated SCC is an indicator of an IMI (Dohoo and Meek, 1982), but the time of sampling might have an influence on the results. Finally, when both the udder health situation and the mastitis prevention and control practices are known, the association between these two can be made.

The specific objectives of this study were, therefore:

- To determine pathogen-specific IRCM per region on Canadian dairy farms and the association of pathogen-specific IRCM with BMSCC and barn type (Chapter 2).
- To determine 1) risk factors associated with IRCM, and 2) risk factors associated with pathogen-specific IRCM, on Canadian dairy farms (Chapter 3).
- To estimate 1) farmer compliance with recommended mastitis preventive management practices on Canadian dairy farms, 2) the herd-level prevalence of contagious mastitis pathogens, and 3) to evaluate the association of certain management practices with contagious mastitis pathogens isolated from bulk tank milk on Canadian dairy farms (Chapter 4).
- To estimate 1) the herd prevalence of contagious mastitis pathogens based on bulk milk from Prince Edward Island dairy farms, 2) determine the association between herd level contagious mastitis pathogens and herd average BMSCC, and 3) investigate the agreement between repeated bulk milk cultures (Chapter 5).
- To determine in the same herds the seasonal pattern over a four-year time period of: 1) BMSCC, 2) elevated ICSCC, 3) IRCM, and 4) pathogen-specific IRCM (Chapter 6).
- To determine: 1) how sampling time affects the sensitivity and specificity of SCC as an indicator of IMI status, and 2) which cells are responsible for the diurnal variation in SCC (Chapter 7).

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CHAPTER 2

INCIDENCE RATE OF CLINICAL MASTITIS ON CANADIAN DAIRY FARMS

by

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2.1 Abstract

In Canada, no nationwide studies of the incidence rate of clinical mastitis (IRCM) have been conducted. Because IRCM and distribution of mastitis causing bacteria can differ geographically, the primary objective of this study was to determine regional pathogen-specific IRCM on Canadian dairy farms. Additionally, association of pathogen-specific IRCM with bulk milk somatic cell count (BMSCC) and barn type were determined. In total, 106 dairy farms in 10 provinces of Canada participated in the study for a period of a year. Participating producers recorded 3,077 cases of clinical mastitis. Mastitis pathogens that were isolated most often were *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus uberis*, and coagulase-negative staphylococci. Overall mean IRCM was 21.8 cases per 100 cow-years in the selected herds and median IRCM was 15.5 cases per 100 cow-years, ranging from 0 to 97.4 per herd. There was no association between BMSCC and overall IRCM, but *E. coli* and culture-negative IRCM was highest in low and medium BMSCC herds. Herds in Ontario and Québec had the highest IRCM and herds in the Western provinces had the lowest IRCM. *Escherichia coli* IRCM was relatively higher in Ontario than in other regions, but *Streptococcus dysgalactiae* IRCM was highest in Québec. Compared with cows in free-stalls, cows in tie-stalls had higher staphylococcal and *Strep. uberis* IRCM, whereas cows in free-stalls had a higher *E. coli* IRCM than cows in tie-stall barns. The focus of mastitis prevention and control programs should differ among regions and be tailored to farms based on housing type and BMSCC.

2.2 Introduction

Despite the fact that much research and effort has been dedicated to mastitis control, it remains a persistent problem and is the most expensive disease of dairy cows (Schepers and Dijkhuizen, 1991). The disease restricts farm net profit both directly and indirectly. Discarded milk and lowered production account for approximately 80% of the costs associated with mastitis (Reneau and Packard, 1991).

Several studies have been conducted in the past to estimate the incidence rate of clinical mastitis (IRCM) in Europe (Schukken et al., 1989a; Barkema et al., 1998b; Peeler et al., 2000; Barnouin et al., 2005; Nyman et al., 2006), North-America (Dohoo et al., 1983; Erskine et al., 1988; Bartlett et al., 1992; Sargeant et al., 1998), Australia (Daniel et al., 1982), New Zealand (McDougall, 1999), and Africa (Kivaria et al., 2006). Distribution of pathogens isolated from clinical mastitis samples differs considerably among countries and even among studies within a country. In Norway for example, *Staphylococcus aureus* is the most frequently isolated bacteria from clinical mastitis samples followed by *Streptococcus dysgalactiae* (Reksen et al., 2006). In Midwest USA low bulk tank SCC (BMSCC) herds coliforms were the most frequently isolated bacteria (Erskine et al., 1988). In Europe, clinical *Klebsiella* mastitis occurs less frequent than clinical *Escherichia coli* mastitis, while in the US *Klebsiella* and *E. coli* are of equal importance (e.g. Barkema et al., 1998b; Roberson et al., 2004). In New Zealand, coliforms are less important as mastitis causing pathogens; *Streptococcus uberis* is the main concern in both clinical and subclinical mastitis (McDougall, 1998).

Canadian studies of the IRCM are scarce and limited historically and geographically (Dohoo et al., 1983; Meek et al., 1986; Sargeant et al., 1998; Van Dorp et

al., 1999; McLaren et al., 2006). A study conducted in 1993-1996 in 32 herds in British Columbia found very low lactational IRCM based on farm records, ranging from 5.6 to 10.5% in first lactation and fifth and greater lactation cows, respectively (Van Dorp et al., 1999). More recent studies in 48 and 65 selected commercial Ontario dairies estimated the lactational IRCM to be 22 and 20%, respectively (Sargeant et al., 1998; McLaren et al., 2006). In an earlier study, also in Ontario, as part of a disease cohort study, lactational IRCM was reported to be 17% (Dohoo et al., 1983). In most studies, no bacteriology of milk samples was performed, except in the study of Sargeant et al. (1998), who isolated *Staphylococcus* spp. other than *Staph. aureus* most often from clinical mastitis samples, followed by coliforms and *Streptococcus* spp.

Geometric mean BMSCC can differ by geographical region (Norman et al., 2000). There is an association between pathogen-specific intramammary infection prevalence in the herd and BMSCC (Roberson et al., 2006) and some studies reported a difference in pathogen-specific IRCM related to BMSCC (Schukken et al., 1989a; Barkema et al., 1998b). Pathogen-specific IRCM, therefore, may differ among geographical regions.

Because no nationwide studies of the IRCM have been conducted in Canada, and because IRCM and distribution of mastitis causing bacteria can differ geographically, the objective of this study was to determine regional pathogen-specific IRCM on Canadian dairy farms. Additionally, association of pathogen-specific IRCM with BMSCC and barn type was determined.

2.3 Materials and Methods

2.3.1 *Herd selection*

In total, 116 dairy herds in all 10 provinces of Canada were purposively selected through either local veterinary practitioners or provincial Canadian Quality Milk Program (http://www.dairyinfo.gc.ca) coordinators. Each practitioner or coordinator selected herds based on preparedness of the producer to participate and their proximity to the study center and each other. Herds participated in the study for a 12 month period between November 2003 and July 2005. All herds provided production and SCC data, except for 3 of 4 herds in Newfoundland, 1 herd in Québec, and 1 herd in Ontario which had never subscribed to milk recording through DHI, and 5 herds that cancelled their DHI services during the study period. In the end, 106 farms were able to provide complete DHI data.

2.3.2 *Sampling*

Participating producers were asked to collect a milk sample aseptically from every quarter that had visible signs of clinical mastitis and to record cow identification, quarter, date, clinical signs such as abnormal milk, abnormal udder (swollen, red, or hard), fever, off feed, teat injury, and the treatment, if the cow was treated. Clinical mastitis was identified by the producer based on clinical signs including abnormal milk or abnormal udder or both. Every producer received a milk sampling package consisting of sample tubes, alcohol pads, latex gloves, instruction sheet, protocol for aseptic collection of milk samples, recording forms, and labels. Milk samples were stored in a freezer on the farm (at approximately -20°C) and collected every 4 to 6 weeks by the

veterinarian or Canadian Quality Milk coordinator, who sent the frozen milk samples on ice-packs by overnight courier to the Atlantic Veterinary College (Charlottetown, Prince Edward Island) for bacterial culture.

A questionnaire was administered on every farm during the study period. The questionnaire was designed with closed questions and semi-closed questions only. Questions were tested on 3 farms and by 3 technicians at the Atlantic Veterinary College to test if they were understood easily and interpret correctly and, where necessary, they were changed and improved. After a final version was decided upon, the questionnaire was translated into French, but no further testing was conducted on this version. All answers were coded and checked upon receiving the questionnaire, entered twice using data-entry software, EpiData Entry (Lauritsen and Bruus, 2006), and both entries were compared to check for errors.

Specific cow and lactation data, such as calving dates, parity, and culling dates, and specific herd data, such as BMSCC and herd size, were obtained from the regional DHI organizations.

2.3.3 *Laboratory analysis*

Bacteriological culture of milk samples was performed according to NMC standards (Hogan et al., 1999). One modification was made using highly selective media for identifying *Streptococcus* spp. as suggested by Zadoks et al. (2005): *Streptococcus* spp. not splitting esculin on a blood agar plate with 0.1% esculin were considered to be *Strep. dysgalactiae*; remaining streptococci were plated on an Enterococcosel® agar (BD Diagnostic Systems, Sparks, MD, USA) and incubated for 24 h at 37°C; streptococci that were not splitting esculin on the Enterococcosel® agar, were

considered to be *Strep. uberis*; the remaining organisms on Enterococcosel® were considered to be Group D *Streptococcus* or *Enterococcus* spp. Ten μL of milk was cultured and the number of colony-forming units of each of the bacterial species was counted. The contagious pathogens *Staph. aureus* and *Strep. agalactiae* were considered to cause an IMI if 1 colony (100 cfu/mL) was isolated (Hogan et al., 1999). Isolation of ≥ 200 cfu/mL of environmental mastitis pathogens (*E. coli*, streptococci other than *Strep. agalactiae*, *Enterococcus* spp., coagulase-positive staphylococci other than *Staph. aureus*, *Klebsiella* spp., *Arcanobacterium pyogenes*, *Serratia* spp., *Pseudomonas* spp., and *Pasteurella* spp.) or ≥ 1,000 cfu/mL of *Corynebacterium bovis*, coagulase-negative staphylococci, yeasts, molds, fungi, or *Bacillus* spp. were considered significant. Milk samples with 3 or more isolates were considered to be contaminated unless *Staph. aureus* or *Strep. agalactiae* were isolated.

2.3.4 *Statistical analysis*

Data were examined for unlikely values; no data were excluded for this reason. All cases of mastitis recorded by the producers were initially used in the analysis. A second or third case of clinical mastitis in the same lactation, regardless of culture result, was considered a new case if there were at least 14 days between the previous and the current case of clinical mastitis.

Association of BMSCC with IRCM was assessed using a negative binomial regression analysis on IRCM separately for the natural logarithm of BMSCC and BMSCC category. Association of IRCM with barn type, province, region, and region corrected for barn type were also analyzed using individual negative binomial regression. Regions were defined as Western provinces, Ontario, Québec, and Atlantic

provinces, in order to have sufficient herd numbers per geographical region. Western provinces consist of British Columbia, Alberta, Saskatchewan, and Manitoba. Atlantic provinces consist of New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland. Based on the geometric mean of monthly BMSCC during the study period, herds were assigned to one of low, medium, or high BMSCC categories: < 150,000, 150,000 - 250,000, and > 250,000 cells/mL, respectively.

Cows were at risk during the time the herd was enrolled in the study. Per lactation, the time at risk, in days, started at calving date, if the cow entered the herd, if the herd entered the study, or if the last mastitis date was more than 14 days ago and ended if the cow had mastitis, died or was culled, the herd left the study, or if the cow started a new lactation. The incidence rate was calculated as the number of mastitis cases per 36,500 days at risk (100 cow-years) in a herd. Incidence rate, time at risk, and overdispersion of the models were assessed as described by Dohoo et al. (2003). All statistical analyses were performed using Intercooled Stata 8.2 (Intercooled Stata for Windows, version 8.2. Stata Corporation, College Station, Texas, USA).

2.4 Results

Ninety-six out of 116 (83%) participating herds completed the questionnaire. Lactating cows were housed in 3 different barn types: 47 free-stalls (49.0%), 43 tie-stalls (44.8%), and 6 straw packs or combination of barn types (6.3%). Free-stall barns were most common in the Western provinces, whereas tie-stall barns were most common in Québec (Fig. 1). The Atlantic provinces and Ontario had approximately equal proportions of tie-stalls and free-stalls (Fig. 1). Average herd size was 106 cows and ranged between 23

and 649 cows (dry and lactating cows). Because a proportional representation of farms over the Canadian provinces was attempted, Québec had the largest proportion of participating herds: 26 (24.5%), whereas only 1 Newfoundland herd (0.9%) participated (Table 1). Geometric mean BMSCC was lowest in the participating Prince Edward Island farms (146,000 cells/mL, Table 1), while participating Manitoba farms had the highest BMSCC (262,000 cells/mL).

Participating producers recorded 3,077 cases of clinical mastitis and submitted 3,024 (98.3% of cases had samples submitted) samples. Mastitis pathogens that were isolated most often were *Staph. aureus*, *E. coli*, *Strep. uberis*, and coagulase-negative staphylococci (Table 2). No bacteria were isolated in 1,324 (43.0%) samples and 260 (8.4%) samples were considered contaminated. *Streptococcus agalactiae* was found in 4 (0.1%) clinical mastitis cases, all of which were retrieved from 1 farm in Québec.

Mean herd IRCM was 21.8 cases per 100 cow-years in the selected herds and median IRCM was 15.5 cases per 100 cow-years, ranging from 0 to 97.4 per herds (Fig. 2; Table 1). Incidence rates were different by province ($p < 0.01$) (Table 1). Ontario had the highest IRCM, 31.2 cases per 100 cow-years, compared to Manitoba where the IRCM was 7.6 cases per 100 cow-years. Compared with other regions, participating herds in Ontario and Québec had the highest mean herd IRCM and the herds in the Western provinces had the lowest IRCM (Fig. 3). During lactation, IRCM was highest in the first week after calving, declined considerably in the second week and had a declining trend towards late lactation and a slight upward trend from week 45

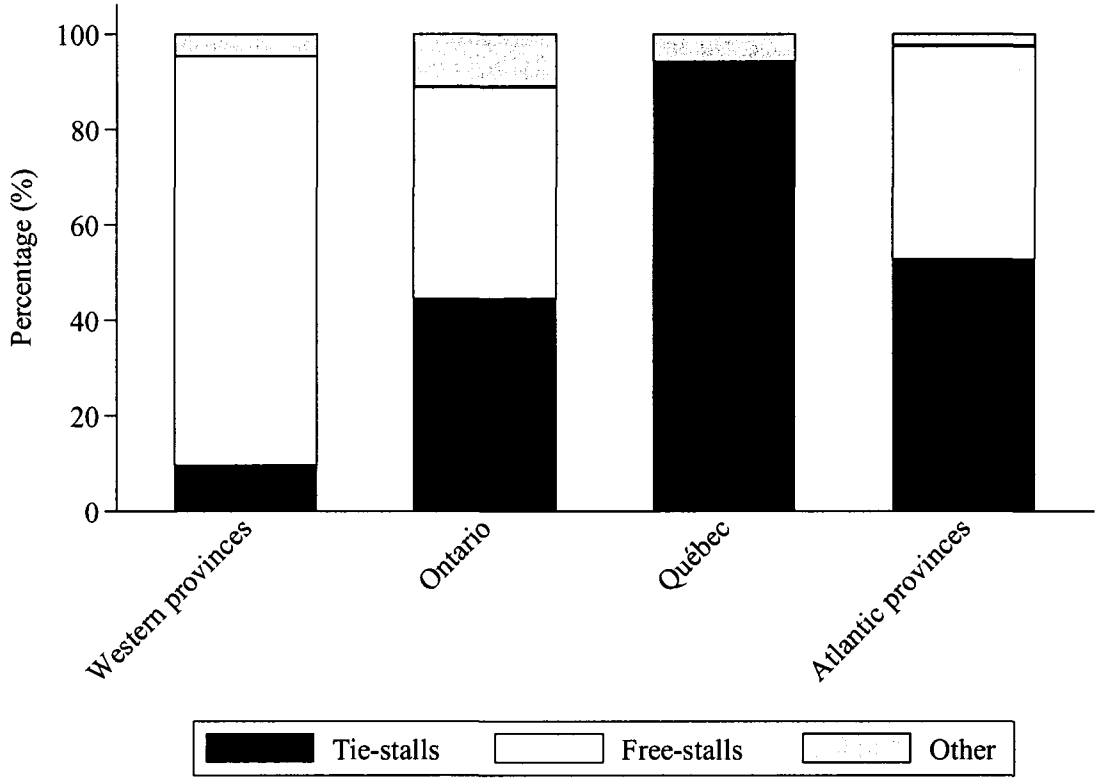

Figure 1. Distribution of lactating cow barn types over the four regions of Canada of the 106 participating herds.

Table 1. Distribution of participating herds and incidence rates of clinical mastitis (IRCM) over Canadian provinces.

Province	Number of herds participating in study	Number of mastitis cases	Total cow years at risk	Predicted mean IRCM per 100 cow-years (SE)	95% CI[1]	Geometric mean BMSCC[2] (x 1,000 cells/mL)	Number of herds as of 31 July 2006[3]	CDC provincial geometric mean BMSCC[3] 2005
British Columbia	8	211	1,426	14.0	8.4 – 23.3	149	641	180
Alberta	10	225	1,210	20.2	12.8 – 31.9	147	720	-[4]
Saskatchewan	5	70	534	13.7	7.1 – 26.6	203	259	-
Manitoba	8	60	1,395	7.6	4.3 – 13.4	262	495	-
Ontario	16	425	1,634	31.2	21.7 – 44.9	205	5,057	214
Québec	26	542	1,895	28.5	21.4 – 37.9	215	7,508	225
New Brunswick	6	82	423	22.8	12.0 – 43.2	190	258	212
Nova Scotia	10	154	1,300	13.8	8.6 – 22.2	160	297	214
Prince Edward Island	16	225	1,275	18.1	12.5 – 26.3	146	246	207
Newfoundland	1	112	377	29.7	7.4 – 119.6	243	41	-
Total	106	2,106	11,469	21.8[5]		184	15,522	-

[1]CI = Confidence interval for the predicted mean IRCM.
[2]BMSCC = bulk milk SCC.
[3]Source: Canadian Dairy Commission (CDC). (http://www.dairyinfo.gc.ca/_english/dff/dff_2/dff_2c_e.htm; last visited January 18, 2007).
[4]Data not available.
[5]Mean IRCM of all herds, not predicted.

Table 2. Distribution of mastitis pathogens in 3,024 submitted milk samples from 113 dairy farms in 10 Canadian provinces.

Pathogen	Frequency	Percentage of samples (%)	Percentage of isolates (%)
Staphylococcus aureus	323	10.5	22.2
Escherichia coli	255	8.3	17.5
Streptococcus uberis	191	6.2	13.1
Coagulase-negative staphylococci	156	5.1	10.7
Klebsiella spp.	132	4.3	9.1
Streptococcus dysgalactiae	121	3.9	8.3
Enterococcus spp.	68	2.2	4.7
Streptococcus spp.	63	2.0	4.3
Yeast	57	1.9	3.9
Arcanobacterium pyogenes	37	1.2	2.5
Bacillus spp.	32	1.0	2.2
Pseudomonas spp.	23	0.7	1.6
Serratia	11	0.4	0.8
Corynebacterium bovis	6	0.2	0.4
Staphylococcus spp.	4	0.1	0.3
Streptococcus agalactiae	4	0.1	0.3
Pasteurella spp.	1	0.0	0.1
Other	51	1.7	3.5
Mixed culture	82	2.7	-
Culture-negative	1,324	43.0	-
Contamination	260	8.4	-
Not sampled but recorded	53	1.9	-

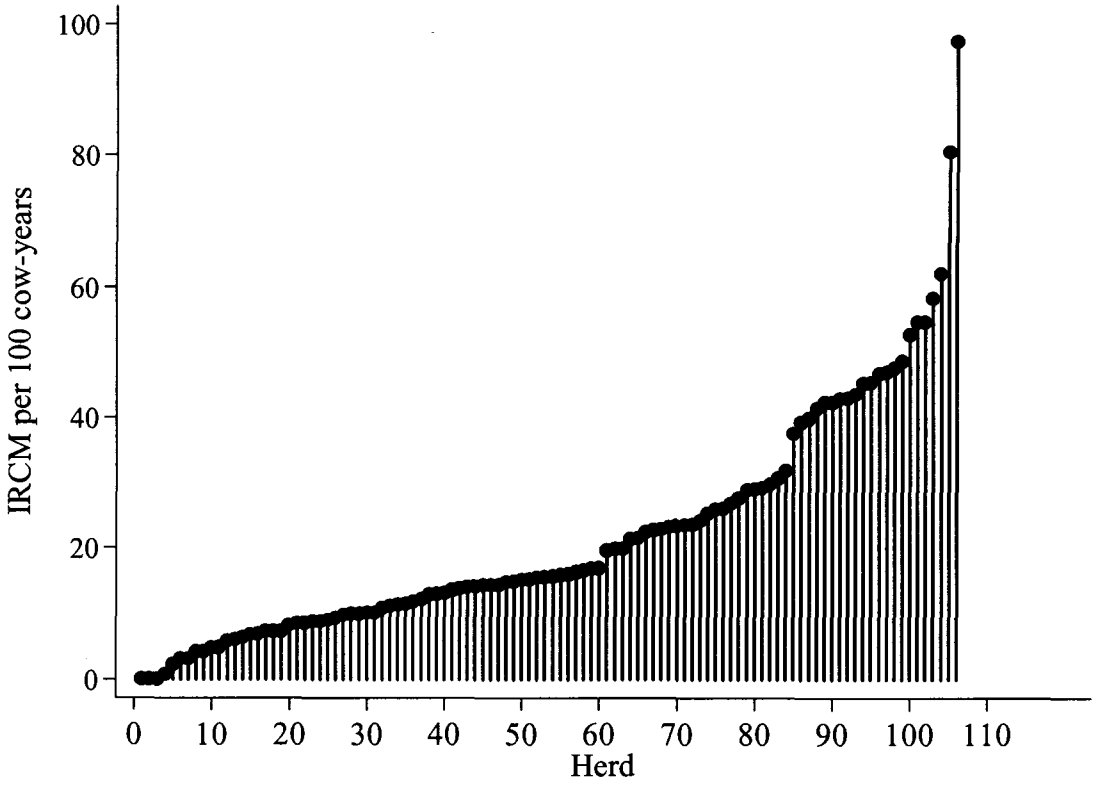

Figure 2. Incidence rate of clinical mastitis (IRCM) in 106 Canadian dairy herds.

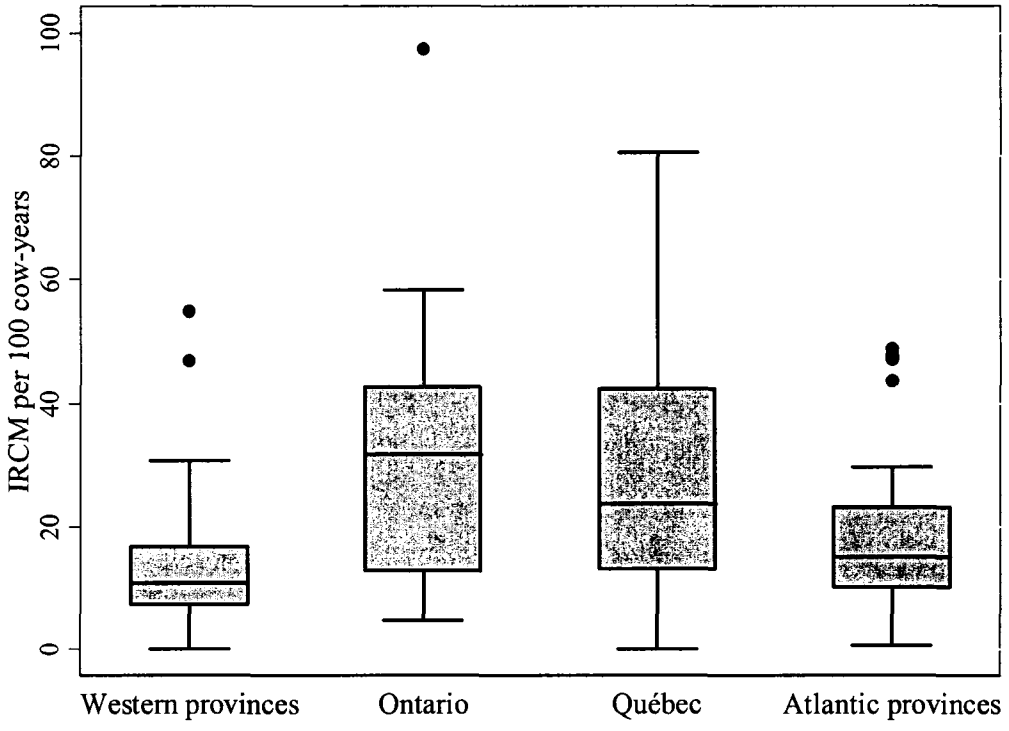

Figure 3. Mean incidence rate of clinical mastitis (IRCM) on 106 dairy farms per region of Canada. (Western provinces, n = 31; Ontario, n = 16; Québec, n = 26; Atlantic provinces, n = 33).

onwards (Fig. 4). Heifers had a higher IRCM than older cows in the first 2 weeks of lactation, between 2 weeks and 45 weeks they had a lower IRCM than older cows, and late to very late in lactation heifers and older cows tended to have similar IRCM (Fig. 4).

No association was found between overall IRCM and BMSCC ($P = 0.58$; Fig. 5). After categorization, the low, medium, and high BMSCC categories consisted of 30 (28.3%), 52 (49.1%), and 24 (22.6%) herds, respectively. Overall IRCM was higher in the low category BMSCC herds than in the high category BMSCC herds. Medium BMSCC herds had higher IRCM than high BMSCC herds, but not significantly (Table 3). In the high BMSCC herds, *Staph. aureus* IRCM was higher (Table 3) than medium BMSCC herds, whereas in low and medium BMSCC herds, *E. coli* and culture-negative IRCM were higher compared with high BMSCC herds (Table 3).

Pathogen-specific IRCM was different by region across Canada. *Escherichia coli* and culture-negative IRCM was relatively higher in Ontario than in other regions, whereas *Staph. aureus* IRCM was highest in Québec (Table 4). *Klebsiella* IRCM was higher and *Streptococcus* spp. IRCM lower in Western provinces than other regions (Table 4). The highest *Strep. dysgalactiae* IRCM were found in Québec in comparison with other regions (Table 4).

Compared with free-stalls, tie-stalls had a higher staphylococcal and streptococcal IRCM, whereas free-stalls had higher *E. coli* and *Klebsiella* spp. IRCM than tie-stall barns (Table 5). Both *Strep. uberis* and *Strep. dysgalactiae* IRCM were highest in other barn types (Table 5).

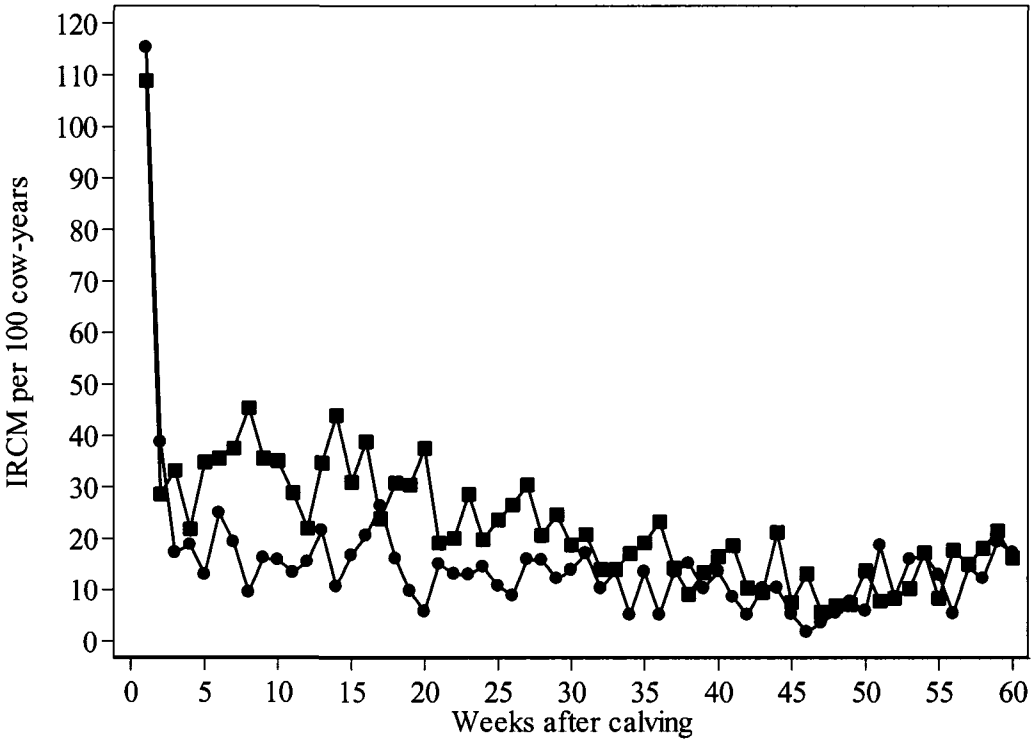

Figure 4. Distribution of incidence rate of clinical mastitis (IRCM) per week after calving for heifers (●) and older cows (■).

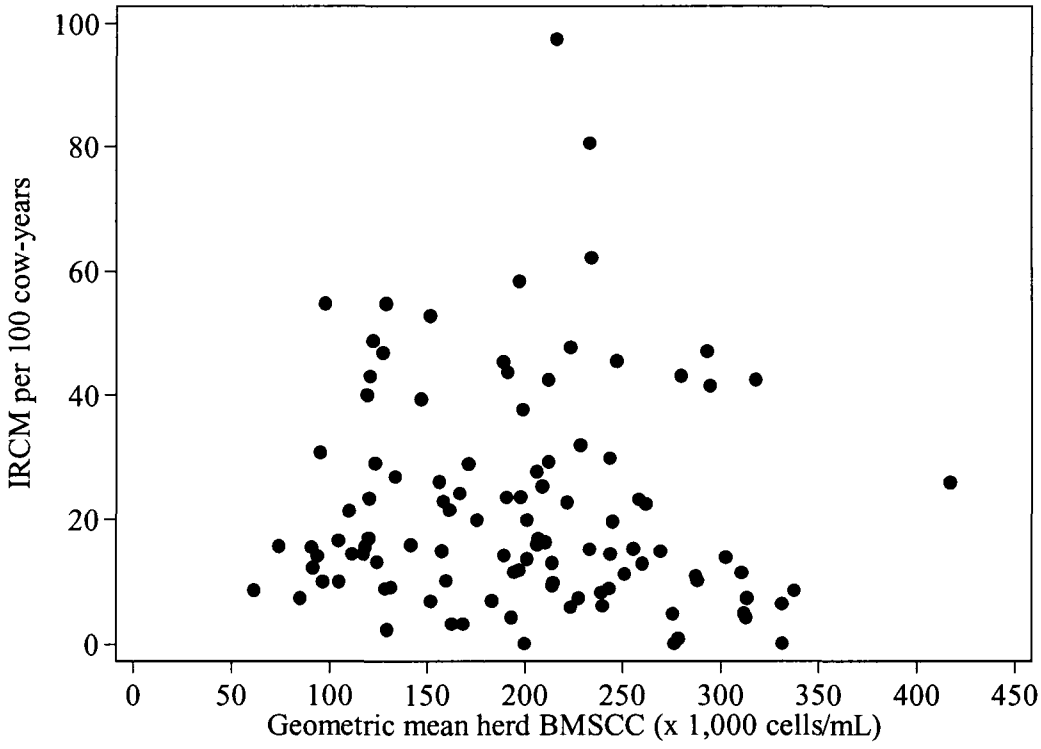

Figure 5. Incidence rate of clinical mastitis (IRCM) versus geometric mean bulk milk somatic cell count (BMSCC) on 106 Canadian dairy farms.

Table 3. Incidence rate of clinical mastitis (IRCM per 100 cow-years) for selected pathogens within 3 bulk milk SCC (BMSCC; x 1,000 cells/mL) categories in 88 Canadian dairy farms.

Pathogen	BMSCC			All herds
	< 150 (n = 30)	151 to 250 (n = 52)	> 250 (n = 24)	
Staphylococcus aureus	2.85[a]	2.33[ab]	4.10[b]	2.89
Escherichia coli	1.98[a]	2.01[(b)]	0.73[a(b)]	1.71
Streptococcus uberis	1.83[a]	1.06[a]	2.01	1.50
Coagulase-negative staphylococci	1.11	1.20	0.99	1.13
Streptococcus dysgalactiae	0.63	0.97	1.14	0.92
Klebsiella spp.	0.49	0.83[c]	0.40	0.64
Streptococcus spp.	0.61	0.48	0.12	0.43
Culture-negative	6.11[a]	6.42[b]	2.23[ab]	5.38
Overall IRCM	22.6[a]	24.1	15.9[a]	21.8

[ab]IRCM on the same row having the same superscripted letters *a* or *b* have a $P < 0.05$, controlled for barn type and region.
[(b)]IRCM on the same row having the same superscripted letter (*b*) have a $P \geq 0.05$ and $P < 0.10$, controlled for barn type and region.

Table 4. Incidence rate of clinical mastitis (IRCM per 100 cow-years) for selected pathogens within region in 106 Canadian dairy farms.

Pathogen	Region				All herds
	Western provinces (n =31)	Ontario (n =16)	Québec (n =26)	Atlantic provinces (n = 33)	
Staphylococcus aureus	1.77^{a}	3.11	4.25^{ab}	2.73^{b}	2.89
Escherichia coli	1.31^{a}	$3.02^{a(b)}$	1.79	$1.39^{(b)}$	1.71
Streptococcus uberis	0.71^{abc}	2.06^{a}	1.49^{b}	2.00^{c}	1.50
Coagulase-negative staphylococci	$0.89^{(b)}$	$1.59^{(c)}$	$1.72^{a(b)}$	$0.67^{a(c)}$	1.13
Streptococcus dysgalactiae	0.24^{ab}	$1.43^{a(c)}$	1.73^{b}	$0.66^{b(c)}$	0.92
Klebsiella spp.	0.96^{a}	0.85	0.26^{a}	0.53	0.64
Streptococcus spp.	0.11^{ab}	0.37	0.62^{a}	0.62^{b}	0.43
Culture-negative	3.92^{ab}	8.30^{a}	6.57^{b}	4.44	5.38
Overall IRCM	14.5^{ab}	32.2^{ac}	28.5^{bd}	18.4^{cd}	21.8

^{abcd}IRCM on the same row having the same superscripted letters *a-d* have a $P < 0.05$.

$^{(bc)}$IRCM on the same row having the same superscripted letters (*b, c*) have a $P \geq 0.05$ and $P < 0.10$.

Table 5. Incidence rate of clinical mastitis (IRCM per 100 cow-years) for selected pathogens within 3 barn types in 88 Canadian dairy farms.

Pathogen	Barn type for lactating cows		
	Tie-stall (n = 43)	Free-stall (n= 39)	Other (n= 6)
Staphylococcus aureus	4.18[(a)]	1.80[(a)]	1.69
Escherichia coli	1.49[a]	2.34[a]	1.37
Streptococcus uberis	2.41[a]	0.75[ab]	2.53[b]
Coagulase-negative staphylococci	1.46	0.70	0.64
Streptococcus dysgalactiae	0.83[(a)]	0.91	1.45[(a)]
Klebsiella spp.	0.47	1.11	0.33
Streptococcus spp.	0.82[a]	0.11[a]	0.00
Culture-negative	5.91	6.03	4.47
Overall IRCM	26.6	19.8	18.6

[ab]IRCM on the same row having the same superscripted letters *a*, *b* have a $P < 0.05$, controlled for region.

[(a)]IRCM on the same row having the same superscripted letter (*a*) have a $P \geq 0.05$ and $P < 0.10$, controlled for region.

2.5 Discussion

The estimated IRCM of 21.8 clinical mastitis cases per 100 cow-years corresponded with the range of IRCM reported by others (Wilesmith et al., 1986; Erskine et al., 1988; Schukken et al., 1989a; Barkema et al., 1998b). The reported IRCM also falls into the range of IRCM found by other authors in Canada. Sargeant et al. (1998) and McLaren (2006) estimates were similar, those of Van Dorp et al. (1999) were much lower Meek et al. (1986) were higher. Considerable ranges of IRCM were found in different studies, varying from 9% per 3-month early lactation period in Australia (Daniel et al., 1982) up to 54.6 cases per 100 cow-years in British dairy herds (Wilesmith et al., 1986). Differences in selection criteria, country, environmental conditions, housing, sampling season, method of data collection, and definition of clinical mastitis undoubtedly contributed to these differences. Methodological differences require caution in comparing IRCM between investigations, but assumed regional differences and barn type differences also underscore that mastitis and milk quality control programs should be tailor-made for specific geographical region and barn type in which cows are housed.

Studies such as this one, where producers select and sample cows with clinical mastitis, have some drawbacks. Firstly the herds were selected for convenience. This method was chosen because producers were asked to take samples and keep records of all clinical mastitis cases. It is likely that this resulted in an overrepresentation of compliant, co-operative producers, or producers with mastitis problems who saw this project as an opportunity to get some free culturing

done. In this study, many farms had a lower BMSCC compared with the average provincial BMSCC as recorded by the Canadian Dairy Commission (Table 1; page 40). These farms represent a different type of management than high BMSCC herds (Barkema et al., 1998a). Producers that were willing to participate were likely to also be keener on reducing IRCM on their farms. This convenience selection could have caused an underestimation of the true IRCM in Canadian dairy herds. On the other hand, the herd selection method provided an opportunity for the participating veterinarians to include farms with mastitis problems in the project. Secondly, detection bias or misclassification bias might have caused underestimation of the IRCM because definition of clinical mastitis might differ among producers. Each herd was provided with the project definition of CM, however, because of the study design, the authors were not able to validate the producers' definition of clinical mastitis. Thirdly, particularly for this study, there was no direct contact between the researcher and the producers, or between the researchers and the veterinarians and Canadian Quality Milk coordinators, which might have curbed motivation for both coordinators and producers to take samples and caused an underestimation of IRCM. In comparison, Barkema et al. (1998b) personally visited every farm every 4 to 6 weeks, and this might have been a reason that the IRCM in his study was higher than in the present study. Although these reasons are major drawbacks in estimating the IRCM, alternatively, visiting every farm to diagnose every case of clinical mastitis would require an enormous financial and manpower effort. Additionally, the researcher's estimation of IRCM might not reflect the producer's perceived IRCM.

Therefore, the data collection methods used in this study were the most feasible and pragmatic approaches under the circumstances.

Detection bias could also have occurred due to severity of clinical mastitis, which is related to pathogens isolated (Gröhn et al., 2005). Producers could be more likely to sample cows with severe clinical mastitis than cows with less severe symptoms. Detection bias could also have occurred among some farms relative to others. Producers who scrutinized foremilk more carefully than other producers (Barkema et al., 1999) could have detected more clinical mastitis cases.

Because veterinarians in Sweden are required to initiate every treatment involving antibiotics, a recent study reported that producers with high veterinary treated IRCM were keener to treat clinical mastitis than producers that had low IRCM (Nyman et al., 2006). Another method, used in a study in British Columbia, Canada, relied on farm records only (Van Dorp et al., 1999) and possibly resulted in a relatively low IRCM. Producers might not record every case of mastitis. They might choose to record only cases of mastitis that were treated, contrary to our study where we instructed producers to take milk samples of every case of clinical mastitis regardless of treatment. Just over half of the cases of mastitis were non-treated in our study (results not shown) and we hypothesize that the cases were mild cases of mastitis and producers normally record fewer of these cases. This is possibly reflected in the higher IRCM we found in our study, because we instructed the producers to take milk samples of every case of mastitis.

The IRCM reported in this study was higher than the IRCM reported in the most recent study in Ontario (Sargeant et al., 1998). Sargeant et al. (1998)

calculated IRCM by using exclusively full 305-day lactations. However, an underestimation of true IRCM could occur here because cows with mastitis are more likely to be culled before the end of lactation (Seegers et al., 2003).

Herds in Ontario and Québec had a higher IRCM than herds in other regions. More than half of the barns in these regions were tie-stalls. The difference in IRCM could be explained by the different management styles directly related to the barn type and different intramammary infection risks associated with barn type. In tie-stall barns it is easier to milk cows with clinical mastitis last or with a separate unit and in free-stall barns wood shavings are used more often as stall bedding material. Cows kept in tie-stall barns also had proportionally more clinical *Staph. aureus* and *Strep. uberis* mastitis compared with those in free-stall barns, whereas cows in free-stall barns have more often *E. coli* and *Klebsiella* mastitis, although the latter one was not statistically different in this study. *Klebsiella* mastitis is associated with using sawdust as bedding material in free-stall barns (Zdanowicz et al., 2004). Similarly, in Scandinavian countries, specifically Norway and Sweden, which have more tie-stall barns, more udders are infected with *Staph. aureus* and *Strep. dysgalactiae* (Østerås et al., 1999). Both *Staph. aureus* and *Strep. dysgalactiae* are considered contagious pathogens (Fox and Gay, 1993) and the spread and prevalence of these pathogens could be attributed to udder preparation procedures in tie-stall barns. Additionally, straw, which is used more often in tie-stalls than in free-stalls, is associated with higher bacteria counts in bedding and a higher IRCM (Zehner et al., 1986; Hogan et al., 1989). Although, *Strep. dysgalactiae* IRCM was

not significantly associated with barn type in our study, it had the highest IRCM in Québec, where most herds are kept in tie-stall barns.

Distribution of barn types in this study was similar to previous research in a random sample of herds (Olde Riekerink et al., 2006a). Tie-stall barns were more common in Ontario, Québec, and the eastern provinces (Olde Riekerink et al., 2006a) and were positively associated with higher IRCM. Western provinces, such as British Columbia and Alberta had few or no tie-stall barns compared with other provinces. Region could therefore be a confounder for differences in IRCM among housing systems. The difference in *Staph. aureus* IRCM was therefore most likely the result of the prevailing barn types per region. A similar situation could be found for *Klebsiella*, and *Strep. uberis* IRCM, which were associated with barn type and not so much with region. By contrast, *Strep. dysgalactiae* IRCM differed significantly per region and seemed not to be associated with either free-stall or tie-stall barns. An explanation for these regional differences could be sought for example in differences in management style, tradition, and herd size. Regional differences in IRCM could therefore only partly be explained by the regional appearance of certain barn types.

Diagnostic tests for mastitis which are described in the Laboratory Handbook on Bovine Mastitis of the NMC (Hogan et al., 1999) to differentiate the most frequently isolated *Streptococcus* spp. are the CAMP, inulin, hippurate, esculin and NaCl tests. A table is provided in this book on how *Strep. agalactiae*, *Strep. uberis*, and *Strep. dysgalactiae* should react. The NaCl test is used to differentiate between streptococci and enterococci (Brown et al., 1983). However, in our experience, this

test was unreliable. Out of a set of 10 *Enterococcus* spp. based on a positive NaCL test, further diagnostics using PCR techniques (Zadoks et al., 2005) identified 7 isolates as *Strep. uberis* (results not published). The tests that are recommended by the NMC are difficult to interpret, because test combinations do not always match and often the diagnoses are "other" *Streptococcus* or *Enterococcus* spp. The authors decided to use Enterococcosel® agar to differentiate *Enterococcus* spp. from non Group D *Streptococcus* spp in addition to the tests recommended by the NMC. Using Enterococcosel® agar, the proportions of clinical mastitis caused by *Strep. uberis*, *Strep. dysgalactiae*, and *Enterococcus* spp. reflected the proportions better, which were expected to be similar to other studies (Barkema et al., 1998b).

Staphylococcus aureus was the most frequently isolated pathogen in clinical mastitis, followed by *E. coli* and *Strep. uberis*. In an earlier study on herd-level prevalences of contagious mastitis pathogens in Canadian dairy herds, *Staph. aureus* was isolated from bulk milk in 74% of a random selection of 291 herds (Olde Riekerink et al., 2006a). Finding *Staph. aureus* most often in milk samples of clinical mastitis was not surprising. Coliforms were most often isolated from cases of clinical mastitis in a study in Ontario (Sargeant et al., 1998), although further differentiation was not performed. *Klebsiella* spp. were the fifth most frequently isolated pathogens. Recently, researchers have suggested that *Klebsiella* incidence is higher in North America than in Europe (Roberson et al., 2004) and that it is an emerging pathogen in North America (Zadoks and Munoz, 2007). However, husbandry in Canadian dairy farms, particularly in Québec, Ontario and Atlantic Canada, differs from US farms and is more similar to the Western-European

situation. As a result, the pathogen distribution of Western Canadian dairy farms is similar to herds with a low BMSCC in the US (Table 4; page 48; Erskine et al., 1988). In the other regions of Canada the distribution is similar to what is found in European studies (Barkema et al., 1998b).

Culture-negative milk samples represented a large part of the milk sample culture results. One reason for a milk sample of a clinical mastitis case to be culture-negative is that those mastitis cases might have been caused by *Mycoplasma*. *Mycoplasma* spp. were not tested for because it requires special growth media. This seems unlikely, however, because incidence of *Mycoplasma* mastitis is generally not so high that it could explain most of the culture-negative samples and the clinical appearance of the culture-negative mastitis cases did not suggest *Mycoplasma* mastitis. Based on a recent study in Prince Edward Island, it would be fair to state that *Mycoplasma* prevalence is most likely low in Canada (Olde Riekerink et al., 2006b). Culture-negative results are often attributed to either *E. coli* (Smith and Hogan, 1993) or *Staph. aureus* (Sears et al., 1990). The distribution of culture-negative IRCM was strikingly similar to *E. coli* IRCM among BMSCC groups (Table 3; page 47), regions (Table 4; page 48) and barn types (Table 5; page 49), whereas *Staph. aureus* IRCM had different distributions. This provides circumstantial evidence that a large proportion of the culture-negative clinical mastitis cases were caused by *E. coli*, and that this pathogen was not present or viable in the milk sample collected (Zorah et al., 1993) or did not survive the frozen storage before culture (Schukken et al., 1989b).

In this study, no linear relationship was found between BMSCC and overall IRCM. This is consistent with some previous research (Barkema et al., 1998b), although other authors have reported an association (Erskine et al., 1988). Consistent with the findings of Barkema et al. (1998b), an association between BMSCC and pathogen-specific IRCM was observed. Barkema et al. (1998b) reported that herds with low BMSCC had a higher *E. coli* and *Strep. dysgalactiae* IRCM and herds with a high BMSCC had a higher IRCM with contagious mastitis pathogens, such as *Staph. aureus*. Similar to Barkema et al. (1998b) there was a higher *Staph. aureus* IRCM in the high BMSCC herds compared with other BMSCC categories and a higher *E. coli* and culture-negative IRCM in the medium and low BMSCC herds compared with the high BMSCC category, indicating a pathogen-specific difference in IRCM between the BMSCC categories. These findings seem to suggest that mainly contagious mastitis pathogens contribute to high BMSCC. If the number of herds in this study had been larger, the additional statistical power might have led to more significant differences in pathogen-specific IRCM between BMSCC categories. The higher BMSCC is most likely caused by increased *Staph. aureus* IRCM. Herds with a high *Staph. aureus* IRCM possibly have more subclinical *Staph. aureus* infections than herds with low *Staph. aureus* IRCM. Higher prevalence of *Staph. aureus* in the herd is likely associated with higher frequency of *Staph. aureus* isolation from consecutive bulk milk samples, which in turn is associated with higher BMSCC (Jayarao et al., 2004; Olde Riekerink et al., 2006b).

2.6 Conclusions

Mean IRCM in selected Canadian dairy herds was 21.8 cases per 100 cow-years, ranging widely among herds. The provinces Ontario and Québec had the highest IRCM, possibly associated with the predominating barn type in those regions being tie-stalls. *Staphylococcus aureus* and streptococcal IRCM were highest in tie-stall barns, whereas *E. coli* IRCM was highest in free-stall barns. The most frequently isolated pathogens in clinical mastitis were *Staph. aureus*, *E. coli*, *Strep. uberis*, and coagulase-negative staphylococci. There was no association between BMSCC and overall IRCM in this study, although pathogen-specific IRCM differed among BMSCC categories. Mastitis prevention and control programs should therefore differ among regions and be tailored towards housing type and BMSCC.

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CHAPTER 3

RISK FACTORS FOR CLINICAL MASTITIS ON CANADIAN DAIRY FARMS

by

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3.1 Abstract

The aim of this study was to determine risk factors associated with the overall and pathogen-specific incidence rate of clinical mastitis (IRCM) on Canadian dairy farms. In total, 116 dairy herds in 10 Canadian provinces were selected through local veterinary practitioners and provincial Canadian Quality Milk Program coordinators. A questionnaire, containing 10 mastitis prevention categories, was administered on every farm. Using negative binomial regression analyses, the association between various risk factors and overall, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, coagulase-negative staphylococci (CNS), and culture-negative IRCM was estimated. Several dry period management practices were associated with overall IRCM, and particularly *E. coli* IRCM: blanket dry cow treatment, average dry period > 60 d, reduced feed energy levels > 7 d before dry-off, and reduction of water intake shortly before dry-off were all associated with a lower *E. coli* IRCM. Herds in free-stall barns had a lower *Strep. uberis* IRCM. Additionally, herds that used sawdust or shavings as bedding material had a lower *Strep. uberis* IRCM compared with herds that used straw. Producers who milked cows with a *Staph. aureus* infection last or with a separate unit during milking had a higher *Strep. dysgalactiae* IRCM, whereas producers that segregated cows with a high SCC, had a higher CNS IRCM, compared with producers that did not follow that practice. Attitudinal risk factors, such as writing down milking procedures (standard operating procedures) were associated with lower overall IRCM. Checking first streams of milk were associated

with higher overall IRCM and more specifically with *Staph. aureus* and *Strep. dysgalactiae* IRCM, most likely because more clinical mastitis was detected. Mastitis control programs in Canada should also take into account the producers' attitude towards mastitis management practices. Also, pathogen-specific risk factors can be quite different, and it is therefore important in mastitis control programs to identify the pathogen that causes problems in a herd.

3.2 Introduction

Mastitis is the most expensive disease on a dairy farm, mainly because of its high incidence and prevalence, cost of treatment, discarded milk, labor, involuntary culling, and loss of potential production (Schepers and Dijkhuizen, 1991). In a nation-wide study, the incidence rate of clinical mastitis (IRCM) in Canadian dairy cows was 22 cases per 100 cow-years at risk (Olde Riekerink et al., 2007a). Risk factors that have been studied and are associated with the incidence rate of clinical mastitis (IRCM) can be divided in the epidemiologic triad of host, environment, and pathogen (Barkema et al., 1999a). Risk factors for IRCM that have been associated with the host include breed of the cow (Schukken et al., 1990; Nyman et al., 2006), high milk production (Schukken et al., 1990; Barnouin et al., 2005; O'Reilly et al., 2006), leaking of milk (Schukken et al., 1990; Peeler et al., 2000; O'Reilly et al., 2006), decreased resistance to infection via teat end callosity (Neijenhuis et al., 2001), and vitamin E and Se deficiency (Erskine, 1993). Environmental risk factors include straw or wood shavings as bedding material in stalls (Rendos et al., 1975;

Bramley, 1984), inadequate ventilation (Schukken et al., 1991b), and high temperature and humidity (Morse et al., 1988; Hogan and Smith, 1997; Hogan and Smith, 2003). Pathogen-related risk factors include transmission method (e.g. milking procedures) and preferred fomite (e.g. sawdust) (Rendos et al., 1975; Fox and Gay, 1993).

Pathogen-specific risk factors for IRCM have been determined (Schukken et al., 1991b; Lam et al., 1997; Barkema et al., 1999a). For example, post-milking teat disinfection (PMTD) is protective for *Staph. aureus,* but increases *E. coli* IRCM for herds going on pasture in summer, whereas in the summer *Streptococcus uberis* IRCM is higher in pastured herds and *E. coli* IRCM increases in confined herds (Olde Riekerink et al., 2007b).

Most of the published studies of the association of management practices with the IRCM were performed in Europe or in the United States (Erskine et al., 1987; Schukken et al., 1990; Barkema et al., 1999a; Peeler et al., 2000; Barnouin et al., 2005). Important factors, such as climate and housing, that may influence IRCM, differ significantly among countries and continents, while in a large countries such as Canada and the United States these factors also differ among regions (Olde Riekerink et al., 2006). Limited studies have been conducted in Canada on IRCM (Meek et al., 1981; Meek et al., 1986), distribution of mastitis pathogens (Sargeant et al., 1998), and pathogen-specific IRCM, (Keefe et al., 1997; Davidson et al., 1992). However, the latter two studies investigated only specific pathogens on isolated populations of farms. Although they provide useful information in their target populations and target pathogen, definitive synthesis of an

up-to-date and valid description of the problem on a broader spectrum of Canadian dairy farms is difficult.

The aims of this study were to determine risk factors associated with non-specific and pathogen-specific IRCM, on Canadian dairy farms.

3.3 Materials and methods

3.3.1 *Herd selection and sampling*

Herd selection has been previously described (Chapter 1). In short, 116 dairy herds in all 10 provinces of Canada were purposively selected through local veterinary practitioners and provincial Canadian Quality Milk Program (http://www.dairyinfo.gc.ca) coordinators. Herds participated in the study for a one year period between November 2003 and July 2005. Participating producers were asked to collect a milk sample aseptically from every quarter that had visible signs of clinical mastitis. Milk samples were stored in a freezer on the farm (at approximately -20°C) and collected every 4 to 6 weeks by the veterinarian or Canadian Quality Milk coordinator, who sent the frozen milk samples on ice-packs by overnight courier to the Atlantic Veterinary College (Charlottetown, Prince Edward Island) for bacterial culture.

3.3.2 *Laboratory analysis*

Bacteriological culturing of milk samples was performed according to the standards of the NMC (Hogan et al., 1999) with a slight modification to the identification of *Streptococcus* spp. as previously described (Chapter 1). From each milk sample, a 10 μL aliquot was cultured. In each of the cultures, the number of colony-forming units of each of the major bacterial species was counted. *Staphylococcus aureus* was considered to cause an IMI if 1 colony (100 cfu/mL) was isolated (Barkema et al., 1998). Isolation of ≥ 200 cfu/mL of *E. coli*, *Streptococcus dysgalactiae*, and *Strep. uberis* were considered significant. Milk samples with 3 or more different isolates, other than *Staph. aureus* or *Strep. agalactiae*, were considered to be contaminated unless *Staph. aureus* or *Strep. agalactiae* was identified, in which case their presence was considered significant and was recorded as such.

3.3.3 *Questionnaires*

A questionnaire was administered on every farm during the study period, by veterinary students on farm, or by phone, by veterinarians, or by mail. The questionnaire was designed with closed questions and semi-closed questions only. Questions were tested on 3 farms and by 3 technicians at the Atlantic Veterinary College to test if they were understood easily and interpret correctly and, where necessary, they were changed and improved. After a final version was decided upon, the questionnaire was translated into French, but no further testing was conducted on this version (Appendix 1 and 2). All answers were coded and checked

upon receiving the questionnaire, entered twice using data-entry software, EpiData Entry (Lauritsen and Bruus, 2006), and both entries were compared to check for errors. A summary of the 10 categories of management practices interrogated in the questionnaire is presented in Table 1.

3.3.4 *Statistical analysis*

Prior to statistical analysis, data were examined for unlikely values; no data were excluded for this reason. All cases of mastitis recorded by the producers were initially used in the analysis. Cows were at risk during the time the herd was enrolled in the study. Per lactation, the time at risk, in days, started at calving date, if the cow entered the herd, if the herd entered the study, or if the last mastitis date was more than 14 days ago and ended if the cow had mastitis, died or was culled, the herd left the study, or if the cow started a new lactation. The incidence rate was calculated as the number of mastitis cases per 36,500 days at risk (100 cow-years) in a herd. Overdispersion was assessed by a likelihood ratio test which tests the overdispersion parameter $\alpha = 0$, as described by Dohoo et al. (2003) and it was assumed that the variance was a constant multiple of the mean. Correlations between overall and pathogen-specific IRCM were calculated. Potential risk factors were screened using a Poisson model with an overdispersion factor, a negative binomial model. All statistical analyses were performed using Stata software (Intercooled Stata for Windows, version 8.2. Stata Corporation, College Station, Texas, USA).

Table 1. Summary of management practices included in the analysis.

I. General Farm and Management	Herd size, type of housing for lactating cows, dry cows, and bred heifers, use of pasture or exercise yard, written goals for udder health performance, heifers and dry cows housed together, stocking density, stocking density feed bunk space
II. Milking procedures	Milking frequency, written milking procedures, training of milkers, number of milkers in the parlour, ratio of female and male milkers, cow per cloth or towel, check of first streams of milk, use of pre-milking teat disinfection, use of gloves, proportion of cows restrained during milking, cows with clinical or subclinical mastitis or with *Staph. aureus* IMI milked separately, automatic teat cup removal, post-milking teat disinfection, fresh feed available after milking
III. Management of clinical cases	All clinical mastitis treated with antibiotics, teat disinfection before treatment, full versus partial insertion, minimum and maximum number of treatments, clinical cases milked outside milking parlour, treated cows marked, vaccination against mastitis, farm-specific treatment plan, proportion of cows culled for mastitis
IV. Dry cow management	Dry cow treatment for all cows, dry cow treatment product, , use teat sealant, proportion of cows teat sealant implemented, mastitis check of dry cows and heifers checked for mastitis, milking frequency reduced before dry-off, average dry period length, feed energy levels reduced before dry-off, water intake reduced before dry-off
V. Subclinical mastitis management	Proportion of cows culled due to high SCC, bacterial culture, producers' definition of high SCC is $\geq$ 250,000 cells/mL
VI. Milking equipment	Number of milking units, vacuum checked daily, equipment checked by dealer or independent technician, stray voltage tested in the last 2 years
VII. Cow comfort and hygiene	Soft stall base (i.e. rubber mat or mattress), stall bedding material, frequency of manure removal, frequency of bedding change , clipping or flaming of udders, tail docking or clipping, availability of maternity pen, sick cows housed in maternity pen, frequency of cleaning bedding in maternity pen

Table 1. *continued.*

VIII. Biosecurity and prevention, and record keeping	Visitors wear boots or protective clothing provided by producer, purchase of heifers or cows in the last year, antibiotic treatment for heifers before calving, knowledge of *Staph. aureus* and *E. coli* outbreaks, record keeping with a computer, using permanent records for mastitis cases, keeps records of bacterial cultures
IX. Nutrition	Total mixed ration fed to the cows, ration balanced > 1 time per y, leftovers fed to dry cows, energy levels adapted to stage of lactation, sugar beet pulp, corn silage, or potatoes are part of the ration, commercial mineral mix fed, supplementation of cows with vitamin E / Selenium, monensin, niacin, yeast, or organic minerals, independent nutritionist, feed company representative, or veterinarian most important for balancing rations, well as water source, water tested for bacteria, water-related bacterial problems in last 2 years
X. Mastitis plan review and communication	Veterinarian or other consultant most important person to review mastitis data, , DHI data checked immediately when it is received, sit down to review mastitis data more than once a month or when bulk milk SCC is higher than 200,000 cells/mL, read literature more than 2 h per week

3.3.5 *Modeling Process*

The model selection process involved 3 steps. First, all single risk factors were screened in a bivariate negative binomial regression model. Variables with a *P*-value ≤ 0.25 were retained for further analysis. Second, within each category of risk factors (Table 1; pages 70 to 71), variables were offered to a negative binomial model using forward stepwise selection. Variables with a *P*-value ≤ 0.10 were kept in the model and were offered to the final model in step 3. During the third step, the final model was constructed by backward stepwise selection using variables from all 10 risk factor categories. Variables with a *P*-value ≥ 0.10 were removed from the model. This *P*-value was chosen because of the relatively low number of herds participating in this study (Barkema et al., 1999a; Barnouin et al., 2005). In the last step, two-way interactions were tested between the main effects that remained in the model. Careful attention was paid to the epidemiologic plausibility of the resulting models. If variables were correlated (correlation coefficient > 0.50 or < -0.50), only the variable with the best fit was included in the model. The goodness of fit of the model was assessed using the Anscombe residuals, standardized deviance residuals, and Cook's influence statistics (Dohoo et al., 2003). The modeling process was repeated for *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, *E. coli*, CNS, and culture-negative IRCM.

3.4 Results

3.4.1 *Descriptive*

Descriptive statistics for the participating herds were previously reported (Chapter 1). In short, the geometric mean bulk milk SCC was 178,000 cells/mL, ranging from 74,000 to 417,000 cells/mL. In total, 3,077 cases of mastitis were recorded by the participating producers. *Staphylococcus aureus*, *E. coli*, *Strep. uberis*, and coagulase-negative staphylococci were most frequently isolated. The overall mean IRCM was 21.8 cases per 100 cow-years and the median IRCM was 15.5 cases per 100 cow-years, ranging from 0 to 97.4. Positive correlations existed among pathogen-specific and overall IRCM (Table 2). Correlation between *Staph. aureus* IRCM and *Strep. uberis* IRCM was strongest, followed directly by correlation between *Strep. dysgalactiae* IRCM and culture-negative IRCM.

3.4.2 *Management Practices and IRCM*

In the first step of the analysis, 36 out of 102 variables were associated with IRCM ($P \leq 0.25$). In the second step of the analyses, the multivariable analysis per category, 19 variables remained associated with IRCM ($P \leq 0.10$). After offering these 19 variables to the final model (1), 9 were associated with IRCM ($P \leq 0.10$) after backward stepwise selection (Tables 3 and 4). No biological plausible interactions were retained ($P \leq 0.10$) in the final step of the analysis. Anscombe residuals were normally distributed (Fig. 1) and the plot of standardized deviance residuals versus the linear prediction did not reveal any obvious pattern (Fig. 2) indicating a good fit of the model. Between 24 and 39 variables were associated

Table 2. Correlations among pathogen-specific incidence rates of clinical mastitis.

IRCM	*Staphylo -coccus aureus*	*Strep. dys- galactiae*	*Strep. uberis*	*E. coli*	CNS[1]	Culture-negative
Streptococcus dysgalactiae	**0.302**[2]					
Streptococcus uberis	**0.578**	-0.026				
Escherichia coli	0.057	**0.213**	-0.023			
CNS[1]	0.128	-0.027	0.074	**0.196**		
Culture-negative	**0.365**	**0.522**	0.142	**0.420**	0.149	
All cases	**0.620**	**0.498**	**0.396**	**0.479**	**0.389**	**0.833**

[1]Coagulase-negative staphylococci.

[2]Correlations in bold were significant ($P < 0.05$)

Table 3. Variables in univariate analyses and offered to final model per pathogen.

Pathogen	Number of variables			Overdispersion factor α in final model	P (α > 0)
	$P \leq 0.25$ in univariate analysis	$P \leq 0.10$ in category analysis	Final model ($P \leq 0.10$)		
Staphylococcus aureus	25	15	6	0.422	< 0.001
Streptococcus dysgalactiae	34	17	7	0.085	0.314
Streptococcus uberis	34	8	3	0.462	0.001
Escherichia coli	24	9	7	0.266	0.005
CNS[1]	34	15	6	0.456	0.002
Culture-negative	39	16	8	0.323	< 0.001
All cases	37	20	9	0.279	< 0.001

[1]Coagulase-negative staphylococci.

Table 4. Final negative binomial regression model for the incidence rate of all cases of clinical mastitis.

Variable	β[1]	SE	*P*	IRR[2]
Intercept	-7.833	0.217	< 0.001	-
Dry cow therapy for all cows	-0.404	0.166	0.015	0.67
Cephapirin benzothiazin is used as dry cow treatment	0.319	0.136	0.019	1.38
Internal teat sealant used at drying off[3]	0.272	0.139	0.050	1.31
First streams of milk checked	0.340	0.140	0.015	1.40
Milking procedures are written down	-0.264	0.133	0.048	0.77
Proportion of cows culled for high SCC	0.034	0.013	0.007	1.03
Heifers purchased in previous year	-0.303	0.142	0.033	0.74
Other consultant (besides veterinarian) important in review of mastitis plan	-0.303	0.149	0.042	0.74
Udder clipped or flamed at least once a year	0.451	0.146	0.002	1.57

[1]β = regression coefficient.
[2]IRR = Incidence Rate Ratio.
[3]Either alone or in combination with other dry cow products.

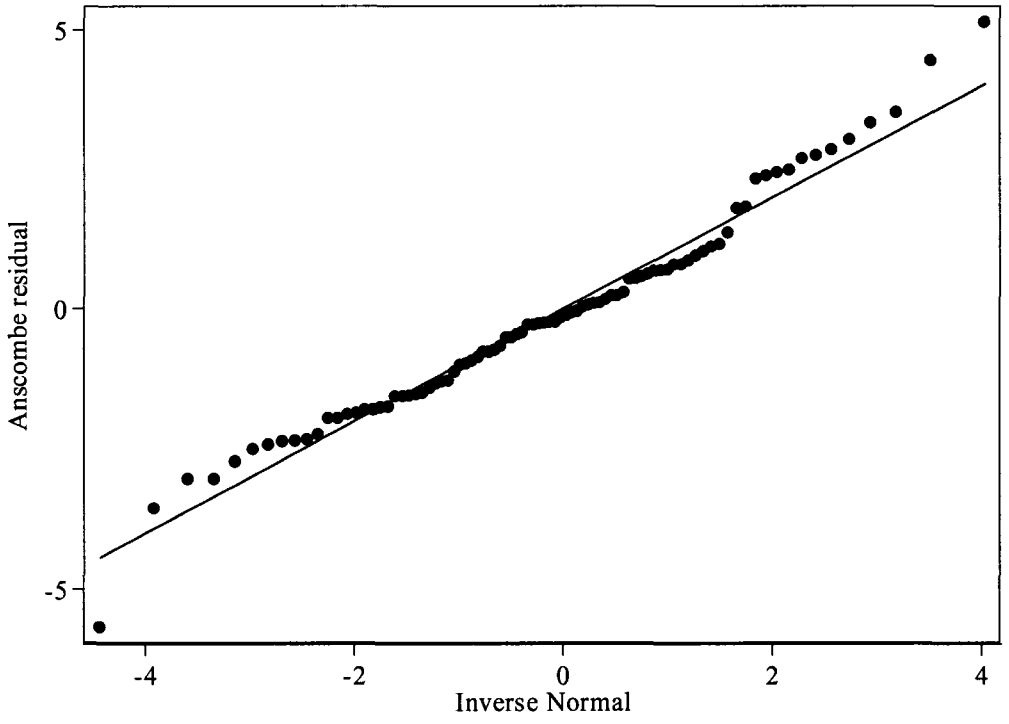

Figure 1. Normal probability plot for Anscombe residuals.

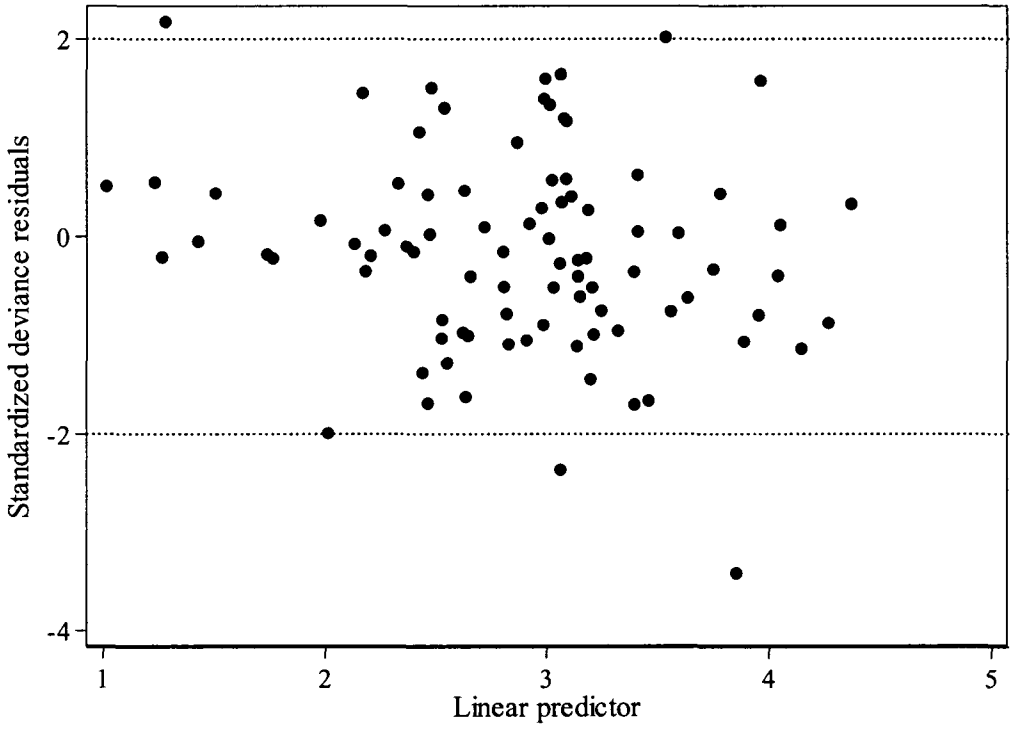

Figure 2. Plot of standardized deviance residuals vs. linear prediction.

with pathogen-specific IRCM ($P \leq 0.25$) in the first step of each analysis (Table 3; page 75). In the second step of the pathogen-specific analysis, between 8 and 17 variables were offered to the pathogen-specific final model (Table 3; page 75) and after the backward stepwise selection only 3 to 8 variables per pathogen were found to be associated with the pathogen-specific IRCM (Table 3, page 75, and Tables 5 to 10). The overdispersion factor α was greater than 0 ($P < 0.01$) for all pathogens, except for *Strep. dysgalactiae*. No biological plausible interactions were retained ($P \leq 0.10$) in any of the pathogen-specific analyses.

Table 5. Final negative binomial regression model for the incidence rate of clinical *Staphylococcus aureus* mastitis.

Variable	β[1]	SE	*P*	IRR[2]
Intercept	-9.453	0.395	-	-
Herd size (lactating cows + dry cows), each cow increase	-0.011	0.002	< 0.001	0.99
Soft stall base, i.e. rubber mat or mattress	1.084	0.309	< 0.001	2.96
First streams of milk checked	0.550	0.234	0.019	1.73
Fresh feed offered immediately after milking	-0.608	0.214	0.005	0.54
Cows are injected with vitamin E / Selenium	0.388	0.236	0.099	1.47
Producer will sit down and review mastitis plan if bulk milk SCC > 200,000 cells/mL	-0.668	0.234	0.004	0.51

[1]β = regression coefficient.
[2]IRR = Incidence Rate Ratio.

Table 6. Final negative binomial regression model for the incidence rate of clinical *Streptococcus dysgalactiae* mastitis.

Variable	β[1]	SE	*P*	IRR[2]
Intercept	-11.118	0.434	-	-
First streams of milk checked	0.647	0.311	0.037	1.91
Pre-milking teat disinfection	-0.646	0.289	0.026	0.52
Vacuum level of milking equipment checked daily	-0.718	0.369	0.052	0.49
Cows with *Staphylococcus aureus* IMI milked last or with a separate milking unit	0.576	0.299	0.055	1.78
Heifers purchased in previous year	-1.128	0.353	0.001	0.32
Cows fed total mixed ration	0.907	0.376	0.016	2.48
Producer spends at least 2 hours per week reading dairy farming literature	-0.600	0.293	0.041	0.55

[1]β = regression coefficient.
[2]IRR = Incidence Rate Ratio.

Table 7. Final negative binomial regression model for the incidence rate of clinical *Streptococcus uberis* mastitis.

Variable	β[1]	SE	*P*	IRR[2]
Intercept			-	
Barn type			< 0.001	
Tie-stall	Ref.[3]	-		-
Free-stall	-1.034	0.303		0.36
Straw-pack barn	0.265	0.424		1.30
Bedding material of the stalls			0.017	
Straw	Ref.	-		-
Sawdust or shavings	-0.463	0.276		0.63
Sand	0.786	0.518		2.19
Producers' threshold for "high SCC" is higher than 250,000 cells/mL	-0.549	0.251	0.029	0.58

[1]β = regression coefficient.
[2]IRR = Incidence Rate Ratio.
[3]Ref. = Reference category.

Table 8. Final negative binomial regression model for the incidence rate of clinical *Escherichia coli* mastitis.

Variable	β[1]	SE	*P*	IRR[2]
Intercept	-10.522	0.684	-	-
Dry cow therapy for all cows	-0.491	0.237	0.038	0.61
Average dry period > 60 days	-0.511	0.213	0.016	0.60
Feed energy levels reduced ≥7 days before dry-off	-0.417	0.238	0.080	0.66
Water intake reduced before dry-off	-2.089	1.130	0.065	0.12
Feed balanced ≥2 times yearly	1.478	0.633	0.020	4.39
High SCC cows milked last or with separate milking unit	-0.371	0.220	0.091	0.69
Vacuum level of milking equipment checked daily	0.426	0.234	0.068	1.53

[1]β = regression coefficient.
[2]IRR = Incidence Rate Ratio.

Table 9. Final negative binomial regression model for the incidence rate of clinical CNS mastitis.

Variable	β[1]	SE	*P*	IRR[2]
Intercept	-11.620	0.506	-	-
Average number of milkers per milking (per person increase)	0.314	0.114	0.006	1.37
High SCC cows milked last or with a separate milking unit	0.951	0.299	0.001	2.59
Bedding in stalls changed at least daily	0.471	0.275	0.087	1.60
Tails docking	-1.225	0.683	0.073	0.29
Cows purchased in previous year	-0.798	0.278	0.004	0.45
Ration supplemented with monensin	-0.585	0.312	0.061	0.56

[1]β = regression coefficient.
[2]IRR = Incidence Rate Ratio.

Table 10. Final negative binomial regression model for the incidence rate of culture-negative clinical mastitis.

Variable	β[1]	SE	*P*	IRR[2]
Intercept	-9.152	0.212	-	-
3 times daily milking	0.597	0.318	0.060	1.82
First streams of milk checked	0.383	0.173	0.027	1.47
More frequent milking of clinical mastitis cases	-0.337	0.189	0.074	0.71
Bedding material of the stalls			0.013	
Straw	Ref.[3]	-		-
Sawdust or shavings	-0.523	0.177		0.59
Sand	-0.312	0.337		0.73
Udders clipped or flamed udders at least once a year	0.371	0.186	0.046	1.45
Tail docking	0.802	0.262	0.002	2.23
Heifers purchased in previous year	-0.333	0.177	0.061	0.72
Ration supplemented with yeast	0.668	0.193	0.001	1.95

[1]β = regression coefficient.
[2]IRR = Incidence Rate Ratio.
[3]Ref. = Reference category.

3.5 Discussion

The IRCM determined in this study varied considerably among selected farms in the Canadian provinces (Chapter 1). The pathogen-specific IRCM also differs among countries (McDougall, 1999; Nyman et al., 2006; Bradley et al., 2007). Therefore, although various studies have been carried out to determine risk factors of clinical mastitis in other countries (Schukken et al., 1990; Barkema et al., 1999a; Peeler et al., 2000; Barnouin et al., 2005; O'Reilly et al., 2006; Nyman et al., 2006), these results cannot always be generalized to the Canadian situation, and recommendations within Canada or the US will need to be tailored to the specific region. In addition, because the herds in this study were not randomly selected, the results of this study cannot automatically be generalized for the Canadian population of dairy herds. In every province veterinarians or Canadian Quality Milk coordinators selected herds to their convenience. This method was chosen because producers were asked to take samples and keep records of all clinical mastitis cases. It is possible that this resulted in an overrepresentation of compliant, co-operative producers, or producers with mastitis problems who saw this project as an opportunity to get some free culturing done, differences between groups of herds of local coordinators.

The nature of risk factors in this study is categorized, although somewhat arbitrarily, into management, attitude, and housing-related risk factors and within these categories the epidemiological triad of host, environment and pathogen.

3.5.1 *Management risk factors*

Several dry period related management practices were associated with overall IRCM, and particularly *E. coli* IRCM; blanket dry cow treatment with antibiotics versus selective or no dry cow treatment with antibiotics, average dry period > 60 d, reduced feed energy levels > 7 d before dry-off, and reduction of water intake shortly before dry-off were all associated with a lower *E. coli* IRCM. Although a recent study concluded that selective dry cow treatment could be the preferred choice based on economic parameters (Huijps and Hogeveen, 2007), blanket dry cow treatment is still the preferred practice because of its proven effect on reduction of new IMI (Berry and Hillerton, 2002b; Bradley and Green, 2004). A large proportion of *E. coli* IMI occurs during the dry period, and the incidence risk depends on dry cow management (Smith et al., 1985; Bradley and Green, 2001). Reducing water and energy levels before dry-off reduces milk production at the time of dry-off. High milk production is associated with an increased risk of clinical mastitis in the following lactation due to slower forming of a sufficient keratin plug (Dingwell et al., 2004; Rajala-Schultz et al., 2005). Longer dry periods were associated with lower *E. coli* IRCM. However, in a study comparing 30 d and 60 d dry periods, no apparent health differences were found (Gulay et al., 2003). To be prophylactic for *E. coli* IMI the antimicrobial used at drying off should have an effect on gram-negative bacteria. Many dry cow treatment formulations do not include that spectrum. The product that was associated with a higher *E. coli* IRCM, cephapirin, does however include gram-negative bacteria in its spectrum. No explanation can therefore be offered for the association found between cephapirin and *E. coli* IRCM.

One of the points of the epidemiological triad, the environment, includes management practices such as grooming udders and tails, docking tails, bedding material, and barn type. Contrary to what was expected, clipping or flaming of udders on a regular basis (at least once a year) was associated with increased overall and culture-negative IRCM. Logically, udders with hair would be expected to collect more dirt than udders without hair. Schreiner and Ruegg (2003) reported an increased risk of IMI caused by major mastitis pathogens for cows with udders characterized as dirty compared with udders characterized as clean. However, it can be argued that udders with hair dry the accumulated dirt faster and plaques of dirt will fall off easier or quicker. Moreover, Silk et al. (2003) found no difference in new IMI in udders with hair and udders with hair removed and suggested that perhaps the current pre-milking preparation techniques, such as pre-dipping, were sufficient to remove or kill bacteria present on the teat.

Tail docking was associated with a higher culture-negative IRCM and a lower CNS IRCM. In previous work it has been shown that tail docking does not have a significant impact on IRCM (Tucker et al., 2001; Schreiner and Ruegg, 2002), and is therefore most likely a proxy for other risk factors.

Streptococcus uberis IRCM was associated with barn type and bedding material. Free-stall barns had lower *Strep. uberis* IRCM than tie-stall barns as is described elsewhere (Chapter 3). Also, stalls with sawdust or shavings had a lower *Strep. uberis* IRCM than stalls with straw. Straw as a bedding material is associated with *Strep. uberis* IMI (Bramley, 1982; Ward et al., 2002). The high *Strep. uberis* IRCM in herds with a sand bedding in this study needs to be interpreted with caution because only 6 herds had sand bedding, but a possible explanation could be that *Strep. uberis* accumulates more in

sand than bedding with sawdust or shavings (Gabler et al., 2001). *Streptococcus uberis* IRCM was also associated with pasture access in a recent study (Olde Riekerink et al., 2007b), but in the current study no significant association with pasture could be found.

3.5.2 *Attitude risk factors*

Attitudinal risk factors are not directly linked to clinical mastitis, but represent merely the attitude of the producer towards prevention and control of mastitis. Some of these factors were associated with the IRCM. Producers who consulted more advisors than the veterinarian and had their milking procedures written down on paper had on average a lower overall IRCM. Producers that are keen to produce high quality milk with the lowest IRCM will more often seek advice of not only their veterinary practitioner but also of other advisors.

Producers who had the milking procedures written down on paper had a lower overall IRCM. Approximately half of the participating farms at the time of the study also participated in the Canadian Quality Milk Program (http://www.dairyinfo.gc.ca). This program requires farmers to write down the milking procedures. Previous research has shown that herds completing a milk quality program reported significant reductions in measures of clinical and subclinical mastitis, reduced bacterial counts in bulk milk, and reduced culling of cows because of mastitis (Rodrigues and Ruegg, 2005). Another explanation could be that early adapters in the Canadian Quality Milk program have developed better udder health practices.

Attitudinal risk factors such as "producer sits down and reviews mastitis plan if BMSCC > 200,000 cells/mL" and "producer spends at least 2 h per wk reading dairy

farm literature" were associated with both a lower *Staph. aureus* and a lower *Strep. dysgalactiae* IRCM (Table 5 and 6) Both risk factors represent the knowledge or stockmanship of the dairy producer and the aggressiveness with which he/she will tackle mastitis problems. Producers that check vacuum levels every day in the milking parlour had an elevated *E. coli* IRCM (Table 8) and a lower *Strep. dysgalactiae* IRCM. Producers that are better managers and have included monitoring of the milking equipment in their routine will more likely be farmers with a low BMSCC (Barkema et al., 1999b). In these low BMSCC herds, *Strep. dysgalactiae* IRCM is lower, but on average *E. coli* IRCM is higher than in high BMSCC herds.

The difference is therefore a difference in attitude in which producers that were classified as "clean and accurate" have a lower BMSCC, and particularly a lower contagious mastitis IRCM than producers that were classified as "quick and dirty" which has been pointed out in previous research by Barkema et al. (1999b). It is unfortunate for these farmers that the knowledge of prevention of clinical *E. coli* mastitis is not as advanced as the tools that are available to prevent contagious mastitis.

3.5.3 *Cause and effect reversal*

Although statistically significant associations between several management practices and risk factors were found, the associations were not necessarily causal. First, when many variables are studied, chances of finding one variable statistically significant just by chance alone is 1 in 20 if statistical significance level (P) was set at 0.05. A variable such as "Producer spends at least 2 h per wk reading dairy farm literature" could have been the result of chance, but is more likely associated with the producers' attitude

or stockmanship. Secondly, confounding factors such as barn type or region for the lactating cows could influence both the outcome as well as other exposure factors.

A management practice that is linked with the risk of infection with contagious pathogens is the segregation of cows infected with these pathogens by either using a separate milking unit or milk these cows last. Producers that segregate cows with a *Staph. aureus* infection during milking, did have a higher *Strep. dysgalactiae* IRCM, whereas producers that segregated cows with a high SCC, had a higher CNS IRCM compared with producers that did not follow that practice. The IRCM of the mainly contagious pathogens *Strep. dysgalactiae* and CNS were both positively correlated with *Staph. aureus* IRCM (Table 2), whereas *Staph. aureus* and *Strep. dysgalactiae* IRCM and prevalence of IMI with these pathogens is higher in high BMSCC herds compared to low BMSCC herds. It is well-known that segregating high SCC cows or cows with a *Staph. aureus* IMI is an efficacious practice to reduce the spread of contagious mastitis pathogens within a herd (Fox and Gay, 1993; Middleton et al., 2001). Therefore, most likely, herds with a high prevalence of subclinical mastitis that also had a relatively high IRCM with contagious pathogens, decided to separate cows with high SCC during milking.

The positive association of checking milk during the udder preparation with IRCM could be explained by the fact that producers that check milk before attaching the milking unit are more likely to discover more cases of clinical mastitis than producers that do not strip. Other authors have found a similar association (Barkema et al., 1999a; Peeler et al., 2000; O'Reilly et al., 2006). On the other hand, while many authors have emphasized the importance of stripping in the udder preparation for milk letdown

stimulation, none have actually shown that stripping is beneficial in reducing IRCM or increasing milk yield (Rasmussen et al., 1990) or have shown no impact in high producing cows (Wagner and Ruegg, 2002). It can even be discussed if stripping in a tie-stall would be beneficial if the rest milk is not removed from the stall, because rests of milk in the stall can act as a nutritional source for bacteria.

Checking first streams of milk was associated with an increased risk of overall, *Staph. aureus*, *Strep. dysgalactiae*, and culture-negative infection. Its association with *Staph. aureus* and *Strep. dysgalactiae* IRCM was stronger than with other pathogens. Both *Staph. aureus* and *Strep. dysgalactiae* often cause chronic mastitis that is most often subclinical (Fox and Gay, 1993). Flare-ups of chronic subclinical mastitis occur frequently. If the milk is not checked during milking than these flare-ups are not detected. It is therefore no surprise that farms which include this practice in their milking routine had a higher IRCM.

Although, vitamin E and Se supplementation improves udder health (Weiss et al., 1990), we found that injecting vitamin E and Se in cows was associated with an increased *Staph. aureus* IRCM. It can be argued that herds that have mastitis problems will adopt management practices such as injecting minerals more readily than herds that do not have problems, because this practice is an investment in both drugs and labor. We hypothesize therefore that injecting cows with vitamin E and Se is more a cure than a prevention practice, and therefore, that herds in areas with vitamin E and Se deficiency, which is associated with a larger risk of intramammary infection, are more likely to supplement.

Preventing cows from lying down and giving the teat canal time to close before bacteria enter the teat can be done by offering fresh feed immediately after milking. This

management practice decreased the *Staph. aureus* IRCM, although we expected this to be more associated with environmental pathogens than contagious pathogens such as *Staph. aureus*, because cows that lie down immediately after milking are more exposed to environmental pathogens.

Internal teat sealants have a proven effect on both the occurrence of new IMI during the dry period and IRCM in the first month after calving (Woolford et al., 1998; Berry and Hillerton, 2002a; Godden et al., 2003; Sanford et al., 2006). The association of this product with an increased overall IRCM is therefore likely the result of implementations by farms that had clinical mastitis problems as an extra measure during the dry cow period to reduce IRCM. Another explanation could be that farmers who chose to use an internal teat sealant expect more from technical measures rather than own management practices.

Herds that purchased heifers had a lower overall, *Strep. dysgalactiae*, and culture-negative IRCM. The IRCM increases with increasing parity (Barkema et al., 1998). Herds that purchased heifers had a slightly younger lactating herd, and likely as a result a lower IRCM. This would of course not be true for herds that are forced to purchase heifers because of large problems with (sub)clinical mastitis.

3.5.4 *Risk factors not in the model*

Variables that originally were associated with IRCM in the univariate models (Table 3), disappeared in the final models. An explanation for this is the relative small number of participating herds (n = 88) and sometimes high correlation among management practices, for example between purchasing heifers and purchasing cows.

It can therefore be argued, similar as was the case with the use of a soft stall base, that these variables are a proxy for other management practices or risk factors such as barn type. Barn type has been associated with increased *Staph. aureus* prevalence and IRCM before (Chapter 1) and producers who house their cows in free-stall barns are more likely to feed their cows fresh feed after milking than other producers, which was the case in this study (results not shown).

3.5.5 *Questionnaire validity and repeatability*

The number of questionnaires that were completed and returned, and the number of producers who collected milk samples over the period of a year, were not as high as in other studies, mainly because of the long distances between the producers and the researchers. Also, having a local coordinator between the producer and the researchers made the communication and motivational distance longer. A study in the Netherlands where the investigators visited the farms personally every 4 to 6 weeks (Barkema et al., 1998) resulted in a return of questionnaires close to 100%.

The validity of the answers of the producers given in the questionnaire will have a certain amount of error, as some authors described errors up to 13% (Schukken et al., 1989c). Validation studies for questionnaires are difficult to obtain, because it is difficult to impossible to retrieve "true" answers, especially with questions regarding attitude or behavior. Additionally, misinterpretation of questions by both the interviewee and interviewer might cause misclassification error. However, in both cases the bias will most likely be towards the null. Some studies have investigated the repeatability of questionnaires and found that repeatability of dichotomous questions is reasonably good

(Schukken et al., 1989c; Scholl et al., 1994). Mistakes will be made also by coding and entering data up to 21% of total of mistakes (Schukken et al., 1989a). One of the most important measure in this study, the number of mastitis cases, was questioned a few times: first the recording of the samples on a supplied barn sheet, second one question in the questionnaire was asking to estimate the approximate number of samples missed during this study, and third a question about the approximate number of cases of clinical mastitis per month. It was expected that those results would match reasonably well. However, in our study we found too much variation between those variables to be able to draw conclusions based on the perceived number of mastitis cases (results not shown). Awareness of disease incidence might be limited among dairy farmers and an estimate therefore of the number of mastitis cases per month on a farm might be far from reliable (Scholl et al., 1994). Some factors in the questionnaire, such as barn type for the lactating cows are quite straight forward and we did not expect too much misclassification in this kind of categories. However, even seemingly straight forward issues like barn type might have a low repeatability (Scholl et al., 1994). Also, answers to questions like "what SCC does a cow have, which is considered to have a high SCC?" might change over time because of discussions with other farmers, or even the questionnaire itself.

3.5.6 *Model fit*

For the analyses of the risk factors a negative binomial model fitted the data best. There was a large variation in IRCM between herds and overdispersion was clearly present (Table 2). Other authors have described similar patterns (Schukken et al., 1989b;

Barkema et al., 1998; Peeler et al., 2000) and Schukken et al. (Schukken et al., 1991a) showed that a negative binomial model best fits data of this nature. The final model was consequently checked using Anscombe residuals which highlights large residuals (Dohoo et al., 2003).

3.6 Conclusion

Several risk factors were associated with overall and pathogen-specific IRCM. Blanket dry cow treatment was, for example, associated with decreased overall, and more specifically, *E. coli* IRCM, whereas herds in free-stall barns had lower *Strep. uberis* IRCM. Attitude risk factors, such as writing down milking procedures were associated with lower IRCM. Checking first streams of milk were associated with higher overall IRCM and more specifically with *Staph. aureus* and *Strep. dysgalactiae* IRCM, because more clinical mastitis will be discovered. Mastitis control programs in Canada should also take into account the producers' attitude towards mastitis management practices. Also, pathogen-specific risk factors can be quite different, and it is therefore important in mastitis control programs to identify the pathogen that causes problems in a herd.

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CHAPTER 4

MANAGEMENT PRACTICES ASSOCIATED WITH THE BULK MILK PREVALENCE OF CONTAGIOUS MASTITIS PATHOGENS IN CANADIAN DAIRY FARMS

by

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4.1 Abstract

The objectives of this study were 1) to estimate compliance with recommended mastitis preventive management practices on Canadian dairy farms, 2) to estimate the herd-level prevalence of contagious mastitis pathogens on Canadian dairy farms, and 3) to estimate associations of certain management practices with the isolation of contagious mastitis pathogens from the bulk tank from Canadian dairy farms. A total of 282 randomly selected farms enrolled in this study, completed a questionnaire and submitted bulk milk samples. Estimated stratified herd-level prevalences of *Streptococcus agalactiae* and *Staphylococcus aureus* in Canada were 4.6% (0.05 – 9.1%) and 73.0% (65.0 – 80.9%), respectively. Highest *Staph. aureus* prevalence was found in Saskatchewan (90%) and lowest prevalence was found in British Columbia (41%). Considerable differences in barn types existed among the provinces; all participating farms in British Columbia had free-stalls cow barns and 91% of farms in Québec had tie-stalls. Post-milking teat disinfection was practised in 96% of the farms and 72% implemented blanket dry cow treatment. Blanket dry cow treatment, believing that a nutritionist is important in mastitis data review, having a feed company nutritionist balance the ration, and having the lactating cow ration balanced at least twice a year were management practices associated with a lower probability of isolating *Staph. aureus*. Having the milking equipment checked by an independent technician at least once a year and rubber mats or mattresses in the stalls were associated with an increased probability of isolating *Staph. aureus* from the bulk tank. Adoption of most of these recommended mastitis management practices is high in Canadian dairy herds. However, significant improvements can still be achieved;

for example, blanket dry cow treatment is practised in only 72% of the dairy herds and in only 50% of the tie-stall herds gloves are worn during milking.

4.2 Introduction

Mastitis is the most prevalent and expensive disease on a dairy farm. Knowledge of the prevalence and distribution of mastitis pathogens as well as risk factors that are associated with the disease are critical to the prevention of mastitis. Bulk tank samples are useful for defining herd infection with pathogens whose main reservoir in the herd is the udder (contagious bacteria), i.e. *Staphylococcus aureus, Streptococcus agalactiae*, and *Mycoplasma* spp. (Oz et al., 1986; Fox and Gay, 1993; Godkin and Leslie, 1993; Jayarao and Wolfgang, 2003). Isolation of contagious mastitis pathogens from the bulk milk is an indication of an infection in one or more cows in the herd (Jayarao and Wolfgang, 2003). Several studies in the United States and Europe have estimated the herd-level prevalence of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp. (Vecht et al., 1989; Khaitsa et al., 2000; Jayarao et al., 2004; Tenhagen et al., 2006). A number of prevalence studies have been conducted in Canada. *Streptococcus agalactiae* prevalence in Canadian bulk milk ranged between 6% in Alberta (Schoonderwoerd et al., 1993) and 43% in Québec (Godkin and Leslie, 1990; Guillemette et al., 1992; Keefe et al., 1997). In a study on Ontario dairy farms, 58 out of 59 bulk milk samples were *Staph. aureus* positive (Kelton et al., 1999). No studies have been conducted on the prevalence of *Mycoplasma* species in Canadian dairy herds since 1972 (Ruhnke et al., 1976), except a study recently conducted on Prince Edward Island,

where a 1.9% herd-level prevalence of *Mycoplasma* spp. was found (Olde Riekerink et al., 2006).

A number of studies in Canada investigated management practices on dairy farms (Spicer et al., 1994; Sargeant et al., 1997). However, these studies did not focus on mastitis management alone and were restricted temporally or geographically. Therefore, compliance to these management practices by Canadian dairy producers is unknown. The combination of knowledge of the prevalence of contagious pathogens and adoption of mastitis management practices and its association will be an important source to give direction to herd-level, province- and nationwide mastitis prevention programs and mastitis research priorities.

Consequently, the objectives of this study were 1) to estimate compliance with recommended mastitis preventive management practices on Canadian dairy farms, 2) to estimate the herd-level prevalence of contagious mastitis pathogens on Canadian dairy farms, and 3) to estimate associations of certain management practices with the isolation of contagious mastitis pathogens from the bulk tank from Canadian dairy farms.

4.3 Materials and methods

4.3.1 *Study population*

Initially, in order to have approximately 300 (68%) positive responses 440 letters were sent to producers that were randomly selected per province from all herds that participated in DHI recording in 2003. Herds were selected from the complete list of farms per province that participated in DHI recording, using computer generated random

numbers. More herds were recruited in Ontario and Québec than other provinces, because these provinces have a larger population of dairy herds than other provinces (Table 1). All participating producers were asked to fill out a questionnaire focusing on mastitis prevention. For each herd, 24 mo of production and SCC data was collected from regional DHI organizations covering the period from October 1, 2004 to September 31, 2006.

4.3.2 *Sample collection*

Samples were collected from the bulk tank by bulk milk haulers who followed a specified sampling protocol. In some cases bulk milk samples were collected by project personnel. Samples were taken from the top of the tank using a clean, sanitized dipper after the milk was agitated for 5 to 10 min (Hogan et al., 1999; Servello et al., 2004). Samples were refrigerated and transported to the provincial dairy laboratory within 24 to 36 h after collection at the farm. In Québec, some samples were taken using autosamplers on milk trucks (Goodridge et al., 2004). Four bulk milk samples per herd over a period of 1 yr were collected by provincial dairy laboratories. Once a laboratory had collected and frozen a batch, samples were sent on ice by overnight courier to the Atlantic Veterinary College (Charlottetown, Prince Edward Island, Canada) for bacteriological culture.

Table 1. Distribution of herds participating in the study and bulk milk somatic cell count (BMSCC) over Canadian provinces.

Province	Number of herds participating in study	Geometric mean BMSCC[1] (x 1,000 cells/mL)	Number of herds as of 31 July 2005[2]	CDC provincial geometric mean BMSCC 2005
British Columbia	34	145	667	180
Alberta	35	167	749	-[3]
Saskatchewan	10	206	278	-
Manitoba	22	239	530	-
Ontario	65	203	5,346	214
Québec	43	249	7,757	225
New Brunswick	26	163	277	212
Nova Scotia	26	200	314	214
Prince Edward Island	28	185	265	207
Total	289	193	16,224[4]	-

[1]BMSCC = bulk milk SCC, determined from October 1, 2004 to September 30, 2006.
[2]Source: Canadian Dairy Commission (CDC) (http://dairyinfo.gc.ca/pdf/farms_shipping_milk.pdf; last visited March 22, 2007).
[3]Not available for 2005.
[4]Includes 41 dairy farms on Newfoundland.

4.3.3 *Questionnaire*

A questionnaire was designed to obtain information about mastitis prevention management practices on Canadian dairy farms. A summary of the points covered under the 10 categories of management practices is presented in Table 2, containing 70 variables in total. The questionnaire was designed with closed questions and semi-closed questions only. The questionnaire survey was conducted on 3 farms and by 3 technicians at the Atlantic Veterinary College to test if the questions were understood easily and interpret correctly and, where necessary, they were changed and improved. After a final version was decided upon, the questionnaire was translated into French, but no further testing was conducted on this version (Appendix 3 and 4). Four weeks after sending the first questionnaire, a postcard reminder was sent to the producers that had not returned the questionnaire. Producers were contacted by telephone, and the questionnaire was sent a second and third time as a reminder. The time between the first and the fourth mailing was 5 mo. All questions were coded and checked on the questionnaire, entered twice using data-entry software (EpiData Entry; Lauritsen and Bruus, 2006), and the duplicate entries were compared to check for errors.

4.3.4 *Laboratory analysis*

Five different culture media were used to detect *Staph. aureus*, *Strep. agalactiae*, and *Mycoplasma* spp. These were 1) blood agar with the addition of 1 g/L esculin; 2) Vogel Johnson agar, a medium selective for staphylococci; 3) modified Edward's medium with the addition of colistin sulphate (5 mg/L) and oxolinic acid (2.5 mg/L), a medium selective for streptococci (Sawant et al., 2002); 4) modified Hayflick's agar, for

Table 2. Summary of management practices included in the analysis.

I. General Farm and Management	Proportion of female young stock ≤ 1 yr and older, type of housing for lactating cows, dry cows, and bred heifers, business mission statement available, written set goals for udder health performance
II. Milking procedures	Number of milkers in the last week in the parlor, udder preparation, dry wipe only, pre-dip and dry, wash, use of disinfectant in water, type of cloth for drying, number of cows per cloth, brand of pre-dip, use of gloves, automatic take-offs, post-milking teat disinfection, applying method, brand of post-dip, milking cows with high SCC, *Staphylococcus aureus* infection, or clinical mastitis last or with a separate unit, access to fresh feed and water immediately after milking
III. Management of clinical cases	Collect milk samples of newly diagnosed clinical mastitis cases, treat all clinical mastitis cases with antibiotics, use of compounded products, full versus partial insertion, teat disinfection, maximal number of treatments, mark the cow, vaccination
IV. Dry cow management	Dry cow treatment (DCT) for all cows, brand of DCT, use of internal teat sealant, proportion of cows with teat sealant, teat disinfection, full versus partial insertion, teat dip or spray after treatment, reduction of energy levels before dry-off, reduction of water intake before dry-off
V. Subclinical mastitis management	Availability of a California Mastitis Test (CMT) on farm, how often is CMT used, take milk samples for bacterial culture
VI. Milking equipment	Brand of milking equipment, check of equipment by equipment dealer or independent technician
VII. Record keeping and analysis	Use of a computer, brand of dairy management software, record system of clinical mastitis, data that are recorded of each case, mastitis data reviewed with veterinarian or other farm consultants, frequency of mastitis data review, review of mastitis data if bulk milk SCC > 200,000 cells/mL
VIII. Cow comfort and hygiene	Stall base soft (i.e. rubber mat or mattress), stall bedding material, frequency of manure removal more than once a day, frequency of bedding change in stalls at least daily, clipping or flaming of udders, clipping or docking of tails
IX. Biosecurity and prevention	Heifers purchased in the last year, cows purchased in the last year, take milk samples of purchased cows, request SCC data prior to purchase, preventive antibiotic treatment for heifers before calving
X. Nutrition	Frequency of balancing the ration, use of independent nutritionist, feed company representative, or veterinarian for balancing rations

the culture of *Mycoplasma* spp., and 5) modified Hayflick's broth for *Mycoplasma* spp. enrichment (Freundt, 1983; Hogan et al., 1999). The detailed procedure has previously been described by Olde Riekerink et al. (2006). *Staphylococcus aureus* was identified by Gram stain, a positive catalase test, α- and β-hemolysis on blood-esculin agar, and a positive tube coagulase test. *Streptococcus agalactiae* was identified by typical appearance on either modified Edward's medium or blood esculin agar, gram-positive staining, a negative catalase test, a positive CAMP test, and a positive latex agglutination test (Remel PathoDx®, Remel Europe Ltd., Dartford, Kent, UK). *Mycoplasma* spp. were identified by the typical fried egg appearance on Hayflick's agar. If *Mycoplasma* spp. were cultured, isolates were sent to the Animal Health Laboratory of the University of Guelph for determination of species by an antibody agglutination method (Rosendal and Black, 1972).

4.3.5 *Data management and statistical analysis*

Prior to statistical analysis, data were checked for unlikely values; no data were excluded for this reason. Herds were considered *Staph. aureus*-positive if *Staph. aureus* was isolated from at least 1 bulk tank sample; all analyses were carried out at the herd level. Weighted province-stratified year prevalence of bulk tank *Staph. aureus* was estimated using provincial numbers of dairy herds as of July 31, 2006 (http://dairyinfo.gc.ca/pdf/farms_shipping_milk.pdf). Adoption of specific management practices between housing systems were compared using Pearson's χ^2 analyses.

Potential management practices that were associated with prevalence of *Staph. aureus* were first screened using a univariate logistic regression model. Variables with *P*-value ≤ 0.25 were offered to the final model. The final model was constructed by

backward stepwise selection. Variables with *P*-value > 0.05 were removed from the model. All possible two-way interactions between remaining significant variables were investigated. The fit of the final model was evaluated using the Pearson goodness-of-fit test.

All statistical analyses were performed using Stata software (Intercooled Stata for Windows, version 8.2. Stata Corporation, College Station, Texas, USA).

4.4 Results

4.4.1 *Descriptive results*

In total, 291 herds were recruited for this study. Nine herds were dropped from the final data because they had insufficient data: they had not completed the questionnaire, had stopped using DHI milk recording services during the study period, or had not submitted at least two sufficient bulk tank milk samples. Mean and median herd size were 78 and 61 lactating and dry cows, respectively. The range was from 19 to 304 cows. Herd geometric mean bulk milk SCC (BMSCC) from October 1, 2004 to September 30, 2006 was 193,000 cells/mL, (95% Confidence Interval (95% CI): 184,000 – 202,000 cells/mL) ranging from 64,000 to 545,000 cells/mL (Table 1; page 108). In total, 1,064 bulk milk samples were cultured and 23, 22, 6, and 1 herds had 1, 2, 3, or 4 missing samples, respectively. The main barn types in which the lactating cows were housed were tie-stall (48%; binomial exact 95% CI: 42 -54%) and free-stall (46%; binomial exact 95% CI: 40 – 52%) (Fig. 1).

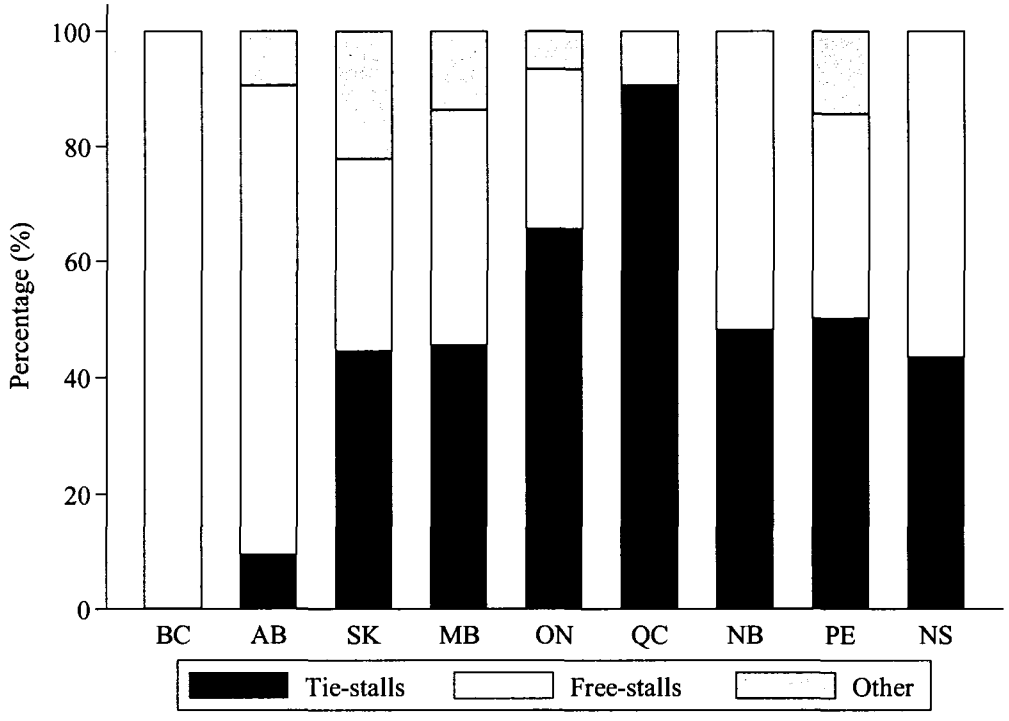

Figure 1. Distribution of barn types in Canada per province (BC = British Columbia, AB = Alberta, SK = Saskatchewan, MB = Manitoba, ON = Ontario, QC = Québec, NB = New Brunswick, PE = Prince Edward Island, NS = Nova Scotia).

Considerable differences existed among the provinces; all participating farms in British Columbia had free-stalls and 91% of farms in Québec had tie-stalls.

4.4.2 *Prevalence of contagious pathogens*

No *Mycoplasma* spp. were isolated from any of the frozen bulk milk samples. *Streptococcus agalactiae* was isolated in 8 (0.75%) of 1,064 samples from 5 (1.8%) herds in Québec (3 herds), Ontario (1 herd), and Prince Edward Island (1 herd). Estimated province-stratified herd-level prevalence of *Strep. agalactiae* in Canada was 4.6% (95% CI: 0.05 – 9.1%).

In total, *Staph. aureus* was isolated in 455 (42%) of 1,064 samples and was isolated in at least 1 bulk milk sample in 204 (72.3%) of 282 herds. Estimated province-stratified herd-level prevalence of *Staph. aureus* in Canada was 73.0% (95% CI: 65.0 – 80.9%) and differed by province ($P = 0.001$) (Fig.2). The highest prevalence was found in Saskatchewan (90%), followed by the Atlantic provinces, New Brunswick (88%), Nova Scotia (88%), and Prince Edward Island (86%). Lowest prevalence was found in British Columbia (41%) and Manitoba (64%) (Fig. 2).

4.4.3 *Adoption of management practices*

Post-milking teat disinfection was done routinely on 96% of the Canadian dairy farms and 72% of farms use blanket dry cow intramammary antibiotics treatment at dry-off (Table 3). Certain management practices were practised more often in free-stalls than in tie-stalls. These practices included pre-milking teat disinfection, wearing latex gloves during milking, and vaccinating cows for mastitis. However, in tie-stall systems mastitic cows were more often milked last or with a separate cluster, and bedding was

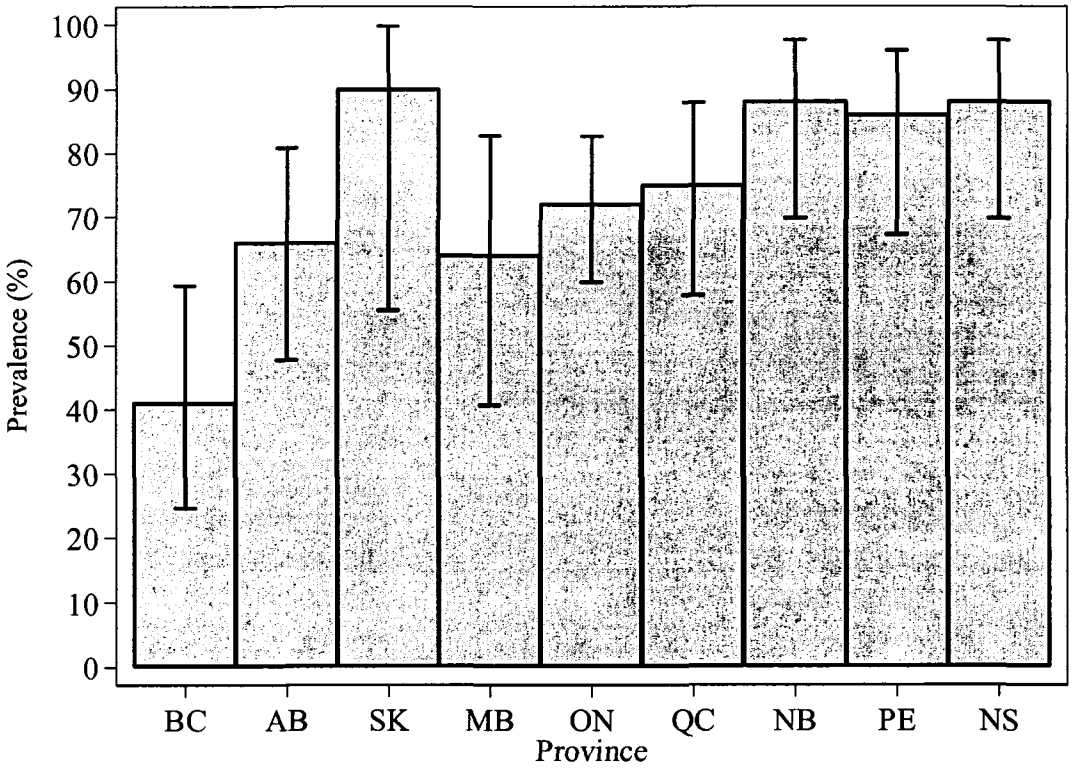

Figure 2. Herd-level prevalence of *Staphylococcus aureus* in Canada per province with 95% binomial exact confidence intervals (BC = British Columbia, AB = Alberta, SK = Saskatchewan, MB = Manitoba, ON = Ontario, QC = Québec, NB = New Brunswick, PE = Prince Edward Island, NS = Nova Scotia).

Table 3. Adoption of mastitis prevention management practices on Canadian dairy farms by barn type for lactating cows in percentages.

Management practice	Tie-stalls (n = 132)	Free-stalls (n= 128)	Other barn types (n= 16)	*P* –value (Pearson χ^2)	All farms
General					
Barn type dry cows					
tie-stall	88.4	10.5	1.2	< 0.01	31.3
free-stall	11.8	85.0	3.2	< 0.01	33.8
other	45.8	41.7	12.5	< 0.01	34.9
Written mission statement	9.2	10.3	25.0	0.15	10.7
Set goals for udder health performance	9.9	9.5	12.5	0.93	9.9
Milking procedures					
> 3 milkers in last week	20.0	28.4	31.3	0.24	24.5
No form of udder preparation before milking	2.3	1.6	0.0	0.77	1.8
Use of pre-milking teat disinfection (pre-dip)	48.1	60.9	56.3	0.12	54.6
Wearing latex or similar gloves during milking	50.0	74.0	56.3	< 0.01	61.5
Use of post-milking teat disinfection (post-dip)	94.6	97.7	87.5	0.13	95.6
Segregate cows with clinical mastitis during milking	86.9	31.5	56.3	< 0.01	59.3
Segregate cows with *Staphylococcus aureus* infection	75.2	21.4	50.0	< 0.01	48.7
Provide fresh feed immediately after milking	88.4	93.0	100.0	0.19	91.2
Management of clinical cases					
Milk samples for culture	15.9	15.6	6.3	0.59	15.2
Treat most of the cases (>50%) with antibiotics	79.6	81.9	87.5	0.71	81.1
Use of compounded products sometimes	32.6	27.6	18.8	0.43	29.4
Mastitis vaccination	12.4	36.2	31.3	< 0.01	24.6
Dry cow management					
All cows get dry cow treatment at drying off	67.9	76.4	75.0	0.31	72.3
Use of an internal teat sealant	20.3	25.4	18.8	0.58	22.6
Partial tip insertion of dry cow tube	68.3	74.6	62.5	0.40	70.9
Reduces energy intake at least 7 d before dry off	22.9	17.2	25.0	0.47	20.4
Subclinical mastitis management					
California Mastitis Test available on farm	68.7	68.8	56.3	0.58	68.0
Milk samples for culture	65.4	67.2	62.5	0.91	66.1

Table 3. *continued*

Management practice	Tie-stalls (n = 132)	Free-stalls (n= 128)	Other barn types (n= 16)	*P* –value (Pearson χ^2)	All farms
Management of records and mastitis data review					
Use of a computer	15.9	50.4	18.8	< 0.01	32.0
Record keeping					
No records	22.3	22.2	6.3	0.32	21.3
Records not permanent	56.2	60.3	81.3	0.15	59.6
Permanent records (computer, cow cards)	21.5	17.5	12.5	0.56	19.1
Veterinarian is important or most important person in reviewing mastitis data	78.6	75.0	53.3	0.10	75.6
Nutritionist is important or most important person in reviewing mastitis data	12.2	20.3	20.0	0.20	16.4
Review mastitis data at least once a month	68.7	70.9	87.5	0.30	70.8
Sit down and review mastitis data if bulk milk somatic cell count > 200,000 cells/mL	13.0	16.5	18.8	0.66	15.0
Cow comfort and hygiene					
Stall base is soft (rubber mat or mattress)	75.8	59.8	-	< 0.01	68.0
Bedding material					
Straw	90.2	30.7	87.5	< 0.01	62.6
Sawdust or shavings	9.1	57.5	12.5	< 0.01	31.6
Sand	0.8	11.8	0	< 0.01	5.8
Manure removal stalls at least twice a day	71.0	73.0	-	0.72	72.0
Bedding changed at least once a day	68.8	23.0	-	< 0.01	46.1
Clip or flame udder hair	75.6	45.3	50.0	< 0.01	60.0
Clip tail hair	62.3	55.1	68.8	0.37	59.3
Dock tails	3.9	17.3	6.3	< 0.01	10.3
Biosecurity					
Purchased heifers in the previous year	34.9	42.7	31.3	0.37	38.3
Purchased cows in the previous year	56.6	43.9	62.5	0.09	51.1
Nutrition					
Balances cows' rations at least twice a year	74.2	93.0	50.0	< 0.01	81.5
Independent nutritionist important or very important in nutrition	18.0	29.7	33.3	0.07	24.4
Feed company representative important or very important in nutrition	69.5	79.7	80.0	0.16	74.9
Veterinarian important or very important in nutrition	29.7	37.5	26.7	0.36	33.2

more frequently changed than in free-stalls. Free-stall herds more often used a computer for herd management than tie-stall herds. In 90% of tie-stalls and straw-pack barns straw was used as the primary bedding material, while wood products were most often used as stall bedding material in free-stalls. Of the five-point mastitis control plan, 120 (43%), 107 (39%), 45 (16%), and 6 (2%) herds implemented 5, 4, 3, and 2 points, respectively.

4.4.4 *Management practices and bulk milk prevalence*

In the first step of the analysis, 25 out of 70 variables were associated with *Staph. aureus* isolation in the bulk milk ($P \leq 0.25$). After offering these 25 variables to the final multivariate model (1), 6 remained associated with *Staph. aureus* prevalence ($P \leq 0.05$). Blanket dry cow treatment, believing that a nutritionist is important in mastitis data review, feed company nutritionist balances the ration, and the ration is balanced at least twice a year, were associated with a lower probability of isolating *Staph. aureus* from the bulk milk (Table 4). Having the milking equipment checked by an independent technician at least once a year and having rubber mats or mattresses in the stalls were associated with an increased probability of isolating *Staph. aureus* in the bulk milk (Table 4). Coefficients did not change considerably after forcing province, barn type, and bedding type into the model for control for possible confounding. The Pearson goodness-of-fit test ($P = 0.94$, $\chi^2 = 23.6$, 36 df) indicated that the model did not fit the data badly.

Table 4. Final logistic regression model of the probability of isolating *Staphylococcus aureus* from the bulk milk.

Variable	β	SE[1]	P	OR[2]
Intercept	1.91	0.59	0.001	-
Ration is balanced at least twice a year	-1.09	0.47	0.023	0.34
Feed company representative is important to very important in balancing rations	-0.89	0.40	0.026	0.41
Nutritionist is (besides veterinarian) important to very important in mastitis plan review	-0.90	0.41	0.030	0.41
Dry cow treatment for all cows	-0.80	0.38	0.034	0.45
Soft stall base, i.e. rubber mat or mattress	2.17	0.34	<0.001	8.78
Milking equipment checked by an independent technician ≥ once yearly	1.12	0.46	0.016	3.06

[1]SE = Standard Error.
[2]OR = Odds Ratio.

4.5 Discussion

This study is the first time that the adoption of mastitis management practices and prevalence of contagious mastitis pathogens has been studied in a nationwide representative stratified random sample of Canadian dairy herds. The results of this study will be useful to determine for which management practices improvements of adoption could be achieved in association with the prevalence of contagious mastitis pathogens in bulk tank milk. When interpreted within the context of the farm's management practices, bulk milk culture, and SCC information provide a basis for evaluating current and potential milk quality and mastitis problems in a herd (Jayarao and Wolfgang, 2003). Only a relatively small number of similar studies have been carried out in other populations (Kirk et al., 1997; Fox et al., 2003).

Staphylococcus aureus was present on nearly all Canadian dairy farms, higher than what has been found on dairy farms in the US (Khaitsa et al., 2000; Jayarao et al., 2004) and New Zealand (Howard, 2006), but similar to what has recently been found in the Netherlands (Sampimon, unpublished data). It is possible that nearly every dairy herd has cows with *Staph. aureus* IMI, which has been suggested by statistical prediction in a herd-level study of *Staph. aureus* prevalence on PEI (Olde Riekerink et al., 2006).

The herd-level prevalence of intramammary infections (IMI) is most accurately determined by culture of quarter milk samples of the whole lactating herd. However, this method is tedious and expensive. Composite samples of the four quarters have, depending on the pathogens involved, an acceptable accuracy to estimate the herd-level prevalence of IMI (Morselt et al., 1995). Bulk milk samples are readily available and

include all cows whose milked is put into the bulk tank. True herd prevalence, defined as the proportion of herds that have *Staph. aureus*-infected udders, can only be determined if the sensitivity and specificity of testing bulk milk samples is known. Boerlin et al. (2003) found a specificity of 100% for the culture method, if *Staph. aureus* was identified by α and β hemolysis on blood agar and a positive coagulase test after 24 h. The sensitivity of a single bulk milk culture is low for *Staph. aureus* and *Strep. agalactiae*, but if consecutive samples are taken, sensitivity can be increased (Godkin and Leslie, 1990; Godkin and Leslie, 1993). True prevalence, as opposed to the reported apparent prevalence, is therefore probably higher in the Canadian national dairy herd assuming that the specificity of the test is close to 100%. Some bias could have occurred by selecting only dairy herds that were participating in some DHI recording program. Herds that were not participating could have had a higher prevalence of contagious mastitis pathogens, the main reason being the inability to monitor individual cow SCC over time and to be able to identify, treat, segregate, or cull cows with chronic infections with contagious pathogens.

The province with the lowest BMSCC, British Columbia, also had the lowest prevalence of *Staph. aureus* in bulk milk. Because British Columbia also had the largest average herd size (results not shown), a dilution effect may have reduced the apparent prevalence somewhat. However, this would imply that the prevalence of *Staph. aureus* at the cow level is lower than in smaller herds. Management practices that are proven tools for the control of *Staph. aureus* IMI are also important tools for reducing BMSCC. Adoption of most of these recommended mastitis management practices is good in Canadian dairy herds. However, significant improvements can still be achieved. For example, blanket dry cow treatment is practised in only 72% of the dairy herds and in

tie-stall herds only 50% wear gloves during milking. Several decades ago, the 5-point mastitis plan was developed (Neave et al., 1969). The focus of this plan was prevention and control of mastitis caused by contagious pathogens. The mastitis-specific pathogens we cultured in bulk milk are all considered contagious pathogens (Fox and Gay, 1993), and two of the three (*Staph. aureus* and *Strep. agalactiae*) were the main target of the 5-point mastitis plan. Currently only 43% of the Canadian dairy farms implement all 5 points of this plan. It is no surprise that the minority of the Canadian dairy farms that does not implement at least 4 points of the 5-point mastitis plan has a higher BMSCC than the farms that do use these proven practices. Previous studies have demonstrated that BMSCC is associated with *Staph. aureus* prevalence in bulk milk (Jayarao et al., 2004; Olde Riekerink et al., 2006). The knowledge to control *Staph. aureus* mastitis in these herds is available; the problem is how to reach and motivate these producers. A monitoring program that cultures bulk milk samples seasonally may be a tool to convince these herds that they have a problem.

In this study, we found *Strep. agalactiae* in less than 1% of the bulk milk samples. Compared to Canadian studies carried out a decade ago, the prevalence of this pathogen has decreased considerably (Godkin and Leslie, 1990; Guillemette et al., 1992; Schoonderwoerd et al., 1993; Keefe et al., 1997). Without exaggeration, *Strep. agalactiae* may be at the brink of eradication in Canada, as is occurring in some North European countries (Pitkälä et al., 2004; Østerås et al., 2006). If countries with less strict BMSCC penalty values would follow the example of European countries and Canada, very likely the prevalence of *Strep. agalactiae* IMI would also decrease in those countries.

One of the goals of this study was to determine whether *Mycoplasma* is an important mastitis-causing pathogen on Canadian dairy farm. After the first round of bulk milk samples was collected, a study was published that found a strong effect of freezing during storage on recovery of *Mycoplasma* in individual quarter milk samples (Biddle et al., 2004). In a pilot study, we found that this may also be the case in bulk milk samples (Olde Riekerink, unpublished). Finding a very low prevalence of *Mycoplasma* in our study, therefore, has limited value if any. This was confirmed by a study carried out on Prince Edward Island where a *Mycoplasma* bulk milk prevalence of approximately 2% was found using fresh samples (Olde Riekerink et al., 2006). We must therefore discard the *Mycoplasma* results from any frozen samples as being inconclusive. To infer that the lack of recovery of *Mycoplasma* from the frozen samples indicates that the pathogen is not present in Canadian bulk tank milk is misleading. Because only fresh milk samples can be used to culture this pathogen, a Canadian study to determine a herd-level prevalence of *Mycoplasma* should be carried out regionally. A polymerase chain reaction on *Mycoplasma* antigen may be another method to use in bulk milk. The accuracy of this method needs to be determined, however.

Two of the 6 risk factors that were associated with isolating *Staph. aureus* from the bulk milk were soft stall bases, i.e. rubber mats or mattresses, and milking equipment checked by an independent technician more than once a year. A cause-effect reversal might have occurred in the latter risk factor. Therefore, most likely, herds which might have problems with *Staph. aureus*, or more general, have a large proportion of the herd with elevated SCC, decided to use an independent technician to check the milking equipment. An explanation for the strong association of soft stall bases with the isolation of *Staph. aureus* from the bulk tank is difficult, but might be that farms which

have rubber mats or mattresses in the stalls, use less bedding material. Teats may come into contact with the stall base more easily and possibly be contaminated by milk leakage of other cows. There would be, therefore, a larger risk for infection with contagious mastitis pathogens. On the other hand, it can be argued that the use of a soft stall base is partly a proxy for other management practices or risk factors such as barn type. However, barn type did not appear to be a confounder in the final analysis.

Two of the risk factors that were associated with a lower *Staph. aureus* prevalence involved the expertise of individuals outside the dairy farm, such as a nutritionist of feed company representative. Producers that buy knowledge this way are more likely to be progressive and willing to invest in this knowledge.

A risk factor that was associated with lower *Staph. aureus* prevalence was blanket dry cow treatment. This is a management practice that is already recommended since the introduction of the 5-point mastitis control plan (Neave et al., 1969) and continues to be associated with lower *Staph. aureus* prevalence. Dry cow treatment is the most efficient method to treat cows with a *Staph. aureus* IMI (Dingwell et al., 2003) and producers that treat all cows with antibiotics at dry-off will therefore keep the herd prevalence low.

It is not surprising that Canadian tie-stall herds use straw for bedding of stalls more often and that free-stall herds use sawdust and wood shavings more often. In a study on a different sample of farms the incidence rate *Strep. uberis* clinical mastitis was three times higher in tie-stall herds compared to free-stall herds, while in the free-stall herds clinical *Klebsiella* mastitis occurred more frequently (Chapter 2). *Streptococcus uberis* mastitis is associated with straw as a bedding (Ward et al., 2002), while wood

products as a bedding material can be a source of *Klebsiella* (Newman and Kowalski, 1973).

Significant improvement of the mastitis prevalence and incidence can only be achieved if herds monitor the mastitis situation within the herd. Permanent record keeping and review of the data together with a specialist are essential in this respect. Use of readily available data such as BMSCC and DHI SCC is not sufficient for this purpose. Because the effect of prevention and control measures is different for the pathogens involved, determination of the distribution of pathogens involved in subclinical and clinical mastitis cases on a regular basis is also necessary. Only a small proportion of the Canadian dairy farms samples clinical mastitis cases (Table 3). These farms essentially implement mastitis prevention practices without knowing what the target pathogens are.

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CHAPTER 5

PREVALENCE OF CONTAGIOUS MASTITIS PATHOGENS IN BULK TANK MILK IN PRINCE EDWARD ISLAND

by

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5.1 Abstract

The purpose of this study was to 1) estimate the herd prevalence of contagious mastitis pathogens in bulk milk from Prince Edward Island (PEI) dairy farms, 2) determine the association between bulk milk culture results and mean bulk milk somatic cell count (BMSCC), and 3) investigate the agreement of repeated bulk milk cultures. Three consecutive bulk milk samples were obtained at weekly intervals from all 258 PEI dairy herds and were cultured using routine laboratory methods. Cumulative prevalence of *Staphylococcus aureus, Streptococcus agalactiae,* and *Mycoplasma* spp. (*M. bovis* and *M. alkalescens*) was 74, 1.6, and 1.9%, respectively. Bulk milk somatic cell count of *Staph. aureus*-positive herds was higher than that of negative herds. Agreement for *Staph. aureus* isolation between 3 consecutive tests was moderate (kappa=0.46). *Mycoplasma bovis* and *Mycoplasma alkalescens* in bulk milk are being reported for the 1st time in PEI ever and in Canada since 1972.

5.2 Introduction

Mastitis is the most prevalent and expensive disease on a dairy farm. Knowledge of the prevalence and distribution of mastitis pathogens is critical to the prevention of the disease. Bulk tank milk culture may be used as a monitoring tool in the control and evaluation of clinical and subclinical mastitis (Jayarao and Wolfgang, 2003). This tool may be usefull while investigating potential milk quality problems on a dairy farm, such as increased bacterial or somatic cell counts (SCC) are being investigated (Jayarao and Wolfgang, 2003; Farnsworth, 1993). Bulk milk culture is a cheap and convenient

method of evaluating milk quality compared with the collection and culturing of individual cow milk samples, and it may be a useful tool for estimating herd level prevalence of contagious mastitis pathogens.

The contagious mastitis pathogens *Staphylococcus aureus, Streptococcus agalactiae*, and *Mycoplasma* spp. reside primarily in the cow's udder; therefore, when they are found in bulk milk, these mastitis causing organisms are strong indicators of the presence of intramammary infections in the herd (González et al., 1986; Fox et al, 2005). *Staphylococcus aureus* is a gram-positive bacterium, can cause subclinical and clinical mastitis in dairy cows and is usually associated with elevated SCC (Pyörälä, 1995; Wilson et al., 1997). *Streptococcus agalactiae* is a gram-positive bacterium, is a contagious obligate parasite of the bovine mammary gland, and most often causes subclinical mastitis and elevated cow SCC (Pyörälä, 1995; Keefe et al., 1997). *Mycoplasma* are pleomorphic bacteria that lack a cell wall, are contagious, and can cause high SCC, and chronic clinical mastitis (Bushnell, 1984; Pyörälä, 1995).

Several studies have been performed to estimate the herd prevalence of *Staph. aureus*, *Strep. agalactiae*, and *Mycoplasma* spp. in the United States and Europe (Greer and Pearson, 1973; Kirk et al, 1997; Khaitsa et al., 2000; Schlegelova et al., 2002; Anderson et al., 2003; Fox et al., 2003). However, only a few studies have been carried out in Canada to estimate the prevalence of contagious mastitis pathogens in bulk milk. The prevalence of *Strep. agalactiae* found in Canadian bulk milk ranged between 6% in Alberta (1993) and 43% in Québec (1992)(Guillemette et al., 1992; Schoonderwoerd et al., 1993). For Prince Edward Island, only Keefe et al. (1997, 1998) have studied herd prevalence of *Strep. agalactiae* and *Staph. aureus*. They found a herd prevalence of 18% and 70%, respectively (Keefe et al., 1997; Keefe et al., 1998). Kelton et al. (1999a,

1999b) found *Staph. aureus* in 58 out of 59 bulk milk samples from Ontario, while 92% of the herds had at least 1 *Staph. aureus* culture-positive cow. In only 1 Canadian study carried out over 30 y ago were *Mycoplasma* spp. found in bulk milk and individual cow milk in Ontario herds: in 33 out of 64 herds *Mycoplasma*-positive and in 182 out of 598 cows (Ruhnke et al., 1976).

Bulk milk SCC (BMSCC) is used worldwide as a measurement for milk quality. Maintaining a low BMSCC benefits both producers and consumers (Emanuelson and Funke, 1991; Barkema et al., 1999; Schaellibaum, 2001). An elevated BMSCC is associated with higher prevalence of subclinical mastitis caused by *Strep. agalactiae* and *Staph. aureus*. Herds may experience a high incidence of clinical mastitis even though the BMSCC remains low (Erskine et al., 1988; Hogan et al., 1989; Schukken et al., 1989). A positive association between herd classification based on BMSCC and the isolation of *Staph. aureus* in bulk milk has been reported (Fenlon et al., 1995; Jayarao et al., 2004).

Currently, reports of a few studies on culture agreement between individual cow or quarter milk cultures are available (Erskine and Eberhart, 1988; Dingwell et al., 2005). However, no studies that estimated the agreement between cultures of consecutive bulk milk cultures have been done.

The objectives of this study were to 1) estimate the herd prevalence of contagious mastitis pathogens in bulk milk from Prince Edward Island dairy farms, 2) determine the association between isolation of contagious mastitis pathogens and herd average BMSCC, and 3) investigate the agreement between repeated bulk milk cultures.

5.3 Material and methods

5.3.1 *Study population*

At the beginning of the study in May 2004, the Prince Edward Island dairy industry consisted of 258 dairy farms. During the sampling period, 1 farm ceased farming. On December 31st, 2003, 193 dairy farms (75%) were enrolled in the milk recording program of the Atlantic Dairy Livestock Improvement Corporation (ADLIC). Among these 193 herds, the average herd size was 59.6 lactating cows, mean annual milk production was 8894 kg/cow (ADLIC, 2003), and the arithmetic mean BMSCC was 245,000 cells/mL (CDC, 2004).

5.3.2 *Sample collection*

Three sets of fresh bulk milk samples were collected from all dairy farms on Prince Edward Island at weekly intervals. Samples were collected from the bulk tank by bulk milk haulers who followed a specified sampling protocol. The milk in the tank was agitated for 10 min before a sample was taken from the top of the tank, using a clean, sanitized dipper (Hogan et al., 1999). Samples were then transported on ice to the provincial dairy laboratory and cultured within 24 to 36 h after collection at the farm. After culturing, SCC was determined within 12 h, except for the 1st set of samples. In the 1st set of samples, ethidium bromide tablets were added as a preservative to the milk and SCC was measured 48 h later.

5.3.3 *Laboratory analysis*

Five different culture media were used to detect *Staph. aureus*, *Str. agalactiae*, and *Mycoplasma* spp. These were 1) blood agar with the addition of 1 g/L esculin; 2) Vogel Johnson agar, a medium selective for staphylococci; 3) modified Edward's medium with the addition of colistin sulphate (5 mg/L) and oxolinic acid (2.5 mg/L), a medium selective for streptococci (Sawant et al., 2002); 4) modified Hayflick's agar, for the culture of *Mycoplasma* spp., and 5) modified Hayflick's broth for *Mycoplasma* spp. enrichment (Rosendal and Black, 1972; Freundt, 1983; Hogan et al., 1999). After mixing the milk on a vortex shaker for 5 s, 50 µL was dispensed by pipette onto the blood esculin agar, Vogel-Johnson agar, and modifled Edward's; 100 µL was dispensed onto Hayflick's agar and into 2 mL of Hayflick's broth. The milk was spread evenly over the plates by a sterile cotton swab and allowed to air dry before incubation.

The Hayflick's broth was mixed on a vortex shaker for a short time and then incubated at 37°C in a moist incubator with 10% CO_2 for 48 h before an aliquot of 100 µL was dispensed onto Hayflick's agar. All Hayflick's agar plates were incubated for 10 d at 37°C in a moist incubator with 10% CO_2. These plates were examined after 48 h and again after 10 d. The blood-esculin agar, Vogel-Johnson agar, and modified Edward's medium were incubated at 37°C aerobically for 48 h. These plates were examined after 24 and 48 h of incubation. *Staphylococcus aureus* was identified by Gram stain, a positive catalase test, α- and β-hemolysis on blood-esculin agar, and a positive tube coagulase test. *Streptococcus agalactiae* was identified by typical appearance on either modified Edward's medium or blood esculin agar, gram-positive staining, a negative catalase test, a positive CAMP test, and a positive latex agglutination test (Remel PathoDx®, Remel Europe Ltd., Dartford, Kent, UK).

Mycoplasma spp. were identified by the typical fried egg appearance on Hayflick's agar. If *Mycoplasma* spp. were cultured, isolates were sent to the Animal Health Laboratory of the University of Guelph for determination of species by an antibody agglutination method (Rosendal and Black, 1972).

Bulk milk SCC (BMSCC) of all samples was determined with an electronic cell counter (Fossomatic Series 400, Foss Electric A/S, Hillerød, Denmark).

5.3.4 *Statistical analysis*

Geometric mean BMSCC per farm was calculated as the exponent of the average natural logarithm (ln) of the 3 BMSCCs. A Student's t test was used to test if the geometric mean BMSCC was different between pathogen-positive and negative farms. One-way ANOVA was used to determine the strength of association of the natural logarithm of BMSCC and the frequency of *Staph. aureus* isolation. The agreement of *Staph. aureus* isolation between 2 consecutive samplings was measured by using kappa, which determines the agreement among tests beyond chance. A kappa between 0 and 0.2 is considered a slight, 0.2-0.4 fair, 0.4-0.6 moderate, 0.6-0.8 substantial, and >0.8 almost perfect agreement (Dohoo et al., 2003). Calculation of geometric mean, ANOVA, and kappa analysis were performed using a statistical software (Intercooled Stata for Windows, version 8.0. Stata Corporation, College Station, Texas, USA).

True herd prevalence, test sensitivity, and test correlation were determined by using maximum likelihood estimation based on a model described by Evers and Nauta (2001), where animal-level prevalence was assumed to vary between herds. The procedure of maximum likelihood estimation determines a set of parameters that makes the observed data most likely (Dohoo et al., 2003). This model was used assuming a

perfect test specificity and constant true herd status, but allowing for conditional dependence between test results. Confidence intervals were computed by the profile likelihood method. For the maximum likelihood estimation the SAS procedure for nonlinear mixed models [PROC NLMIXED] (The SAS system for Windows, version 8.02, SAS Institute, Cary, North Carolina, USA) was used.

5.4 Results

In total, 773 samples were examined, missing 1 sample in the 3rd week. Reading for *Mycoplasma* spp. could not be done on day 10 for 31 samples due to overgrowth by other organisms. *Staphylococcus aureus* was isolated in bulk milk from 191 (74%) dairy farms (Table 1). In every sampling week, *Staph. aureus* was isolated from at least 135 (52%) the samples. Eighty-six (33%) farms tested positive for *Staph. aureus* on every bulk milk sample (Table 2). *Streptococcus agalactiae* was isolated at least once in samples from 4 (1.6%) farms. A *Mycoplasma* sp. was isolated at least once in samples from 5 (1.9%) farms (Table 2). Species determination of in cultures from these 5 farms revealed 2 species, *Mycoplasma bovis* and *Mycoplasma alkalescens*. *Mycoplasma bovis* was found on 2 farms in 1 sample, on 2 farms in 2 samples. *Mycoplasma alkalescens* was found on 1 farm in 1 sample. *Mycoplasma* spp. were never found in 3 consecutive samples on 1 farm (Table 2).

The model in the maximum likelihood procedure that fitted best the data for *Staph. aureus* consisted of a herd prevalence of 100% (95% CI: 80 – 100), a test sensitivity of 54% and a rho of 0.46 (between test correlation).

Table 1. Proportion of Prince Edward Island bulk milk samples (n=258) that were culture-positive for contagious pathogens in 3 consecutive weeks.

	Week 1	Week 2	Week 3	Cumulative prevalence
	Herds (%)	Herds (%)	Herds (%)	Herds (%)
Staphylococcus aureus	135 (52.3)	141 (54.8)	142 (55.3)	191 (74.0)
Streptococcus agalactiae	3 (1.2)	3 (1.2)	1 (0.4)	4 (1.6)
Mycoplasma spp.	5 (1.9)	1 (0.4)	1 (0.4)	5 (1.9)[1]

[1] *Mycoplasma bovis* 4 herds, *Mycoplasma alkalescens* 1 herd.

Table 2. Frequency of contagious pathogens, isolated 0, 1, 2 or 3 times (out of 3 times) in the successive milk samples in a study of 258 Prince Edward Island dairy herds.

	0 out of 3 times Herds (%)	1 out of 3 times Herds (%)	2 out of 3 times Herds (%)	3 out of 3 times Herds (%)
Staphylococcus aureus	67 (26.0)	52 (20.2)	53 (20.5)	86 (33.3)
Streptococcus agalactiae	254 (98.4)	2 (0.8)	1 (0.4)	1 (0.4)
Mycoplasma spp.	253 (98.1)	3 (1.2)	2 (0.8)	0

Farms that had at least 1 bulk tank sample positive for any of the contagious pathogens had a geometric mean BMSCC that was 34,700 cells/mL than the counts from farms that had no pathogens isolated (Table 3) (P=0.006). No difference in BMSCC was found between the 5 *Mycoplasma*-positive and the negative herds, or between the 4 *Strep. agalactiae*-positive and the negative herds (P>0.5). The BMSCC of *Staph. aureus*-positive herds was 39,700 cells/mL higher than that of negative herds (P=0.001).

The BMSCC increased with increasing frequency of *Staph. aureus* isolation (Table 4). *Streptococcus agalactiae* and *Mycoplasma* spp. were not included, because the number of *Strep. agalactiae* and *Mycoplasma*-positive farms were 4 and 5, respectively, and therefore too low from which to draw conclusion.

Kappa for isolation of *Staph. aureus* between week 1 and week 2, between weeks 2 and 3, and between weeks 1 and 3 was 0.42, 0.49, and 0.46, respectively. The combined agreement between the 3 tests gave a kappa value of 0.46. All kappa values indicated a moderate agreement.

Table 3. Association of isolation of any pathogen with average bulk milk somatic cell count (BMSCC).

Pathogen	Mean BMSCC (x1000 cells/mL)		Difference (x 1000 cells/mL)	P-value
	Never isolated (# herds)	Isolated 1 or more times (# herds)		
Staphylococcus aureus	129 (67)	169 (191)	39.7	0.001
Streptococcus agalactiae	157 (254)	177 (4)	20.0	0.69
Mycoplasma spp.	158 (253)	137 (5)	-20.5	0.60
Any contagious pathogen	132 (64)	167 (194)	34.7	0.006

Table 4. Mean bulk milk somatic cell count (BMSCC) in relation with frequency of *Staphylococcus aureus* isolation.

Frequency of *Staph. aureus* isolation	No. Herds	Geometric mean BMSCC (x 1000 cells/mL)			
		Geometric mean	95% CI	Minimum	Maximum
0 (out of 3)	66	129	112 – 148	16	462
1 (out of 3)	51	151	129 – 177	48	537
2 (out of 3)	53	156	134 – 183	56	487
3 (out of 3)	87	188	167 – 213	47	607

5.5 Discussion

The apparent *Staph. aureus* herd level prevalence was in agreement with earlier studies in North America and Europe, where herd level prevalence ranged from 31% to almost 100% (Sischo et al., 1993; Kelton et al., 1999b; Khaitsa et al., 2000; Schlegelova et al., 2002; Jayarao et al., 2004). Prevalence of *Mycoplasma* spp. has been reported for the 1st time on Prince Edward Island, and in Canada for the first time since 1972. However, Canadian laboratories have cultured *Mycoplasma* spp. repeatedly from milk samples.

True herd prevalence, defined as the proportion of herds that have *Staph. aureus*-infected udders, can only be determined if the sensitivity and specificity of testing bulk milk samples is known; therefore, these parameters have to be determined or estimated. For isolates retrieved from bovine mastitis cases, Boerlin et al. (2003) found a specificity of 100% for the culture method, if *Staph. aureus* was identified by α and β hemolysis on blood agar and a positive coagulase test after 24 h. Therefore, in the statistical approach for the true prevalence, we considered the specificity of our method to be 100%. Another study also reported a high specificity for *Staph. aureus* of 93% (Bartlett et al., 1991). Allowing for a lower specificity, the estimated true prevalence would be over-estimated. In the 14-day sampling period, herds could go from a truly negative to a truly positive status for *Staph. aureus* or vice versa. The authors considered it to be unlikely that the infection status of a herd for *Staph. aureus* would have changed in that period.

The *Strep. agalactiae* prevalence of 1.6% confirmed a trend of declining prevalence of this pathogen has declined on Prince Edward Island from 18% in 1994 (Keefe et al., 1997). Herd level prevalence of *Strep. agalactiae* has decreased

considerably over the last years (Keefe, 1997; Pitkälä et al., 2004). Keefe et al. (1997) reported a herd prevalence of 18% on Prince Edward Island in a study performed in 1994. In the current study, the *Strep. agalactiae* herd prevalence appears to be reduced by a factor 10 since 1994. However, Keefe et al. (1997) used a more sensitive method than the standard method recommended by the NMC: in addition to modified Edward's medium, they used modified group B streptococcal (GBS) medium. Sawant et al. (2002) found in a media comparison that modified Edward's medium with the addition of colistin sulphate (5 mg/L) and oxolinic acid (2.5 mg/L) had a sensitivity and specificity of 100%. However, the study was under laboratory conditions, used selected streptococci, and spiked the milk samples. A few authors have estimated *Strep. agalactiae* sensitivities from a single bulk milk sample under field conditions: Godkin and Leslie (1993) found a bulk milk sensitivity of 21% for *Strep. agalactiae* and Bartlett et al. (1991) found a sensitivity of 35%. Both sensitivities were estimated with single bulk milk samples and compared with individual cow composite and quarter samples, respectively. Sensitivity would have been higher if multiple bulk milk samples had been taken. The true prevalence of *Strep. agalactiae* in this study is probably higher than estimated. With a 21% sensitivity and assuming a specificity of 100%, the true prevalence would not estimated to be higher than 7.5%.

For the last 30 y, no Canadian studies have been performed to determine herd level prevalence of *Mycoplasma* spp. Recent US studies, however, suggested that 1% to 6% of the dairy herds had at least 1 cow with *Mycoplasma*-induced mastitis (Jasper et al., 1979; Kirk et al., 1994; Kirk et al., 1997; Fox et al., 2003). Sampling bulk tank milk only a single time may give an underestimation of the prevalence, due to intermittent shedding (Kirk et al., 1994); therefore, multiple sampling should be performed (Jasper et

al., 1979). Sensitivity of a single culture of bulk milk samples for *Mycoplasma* spp. ranges from 33% to 59% (Guterbock and Blackmer, 1984). The *Mycoplasma* spp. that were found in this study are both pathogenic and can cause mastitis (Guterbock and Blackmer, 1984; Kirk et al., 1997). *Mycoplasma bovis* is considered the most pathogenic *Mycoplasmas* sp. (Guterbock and Blackmer, 1984).

In this study, there was a significant association between the isolation of *Staph. aureus* and the mean BMSCC. This is in agreement with other studies (Fenlon et al., 1995; Barkema et al., 1999; Jayarao et al., 2004). The frequency of isolation of *Staph. aureus* (amount of times it was isolated from the 3 samples) has been shown to be significantly associated with the BMSCC. Jayarao et al. (2004) have shown similar associations in a recent study in Pennsylvania. The BMSCC and isolation of *Strep. agalactiae* were not significantly associated in their study, but only 4 farms were considered positive. Other studies have shown that isolation of *Strep. agalactiae* in bulk milk is highly correlated with high BMSCC (Erskine, 1990; Keefe, 1997). However, the presence of certain strains of *Strep. agalactiae* is not correlated to high BMSCC. A possible explanation is that the bulk tank milk was contaminated with human strains of *Strep. agalactiae* (Zadoks et al., 2005).

The isolation of *Mycoplasma* spp. and mean BMSCC were not significantly associated in this study. The main reason is most likely that the number of *Mycoplasma*-positive farms was very low. Fox et al. (2003) have previously reported an association. One explanation could be that the isolation of *Mycoplasma* spp. in bulk tank milk is not related to the number of shedding cows (González et al., 1986). Other explanations could be the low sensitivity of bulk milk culture or that bulk milk was contaminated with *Mycoplasma* spp.

The test agreement of repeated bulk milk cultures was calculated to be moderate. This indicates that the culture of 1 bulk milk sample is not sufficient to correctly classify a herd's *Staph. aureus* infection status.

The apparent herd level prevalence of *Staph. aureus* infection in Prince Edward Island dairy herds is high and similar to that in previous research done elsewhere. As estimated by 3 bulk milk cultures done at weekly intervals, at least 74% of Prince Edward Island herds likely have at least 1 cow with udder infection due to *Staph. aureus*. The prevalence of *Strep. agalactiae* has decreased and is low. Two species of *Mycoplasma* were cultured from Prince Edward Island herds for the first time. Reduction of *Staph. aureus* and *Strep. agalactiae* infections is a useful tool in the reduction of BMSCC on a dairy farm. The agreement between repeated *Staph. aureus* cultures from bulk milk samples with weekly intervals is moderate and, therefore, for reliable determination of the presence of *Staph. aureus*, more than 1 bulk milk sample is needed.

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CHAPTER 6

THE EFFECT OF SEASON ON SOMATIC CELL COUNT AND THE INCIDENCE OF CLINICAL MASTITIS

by

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6.1 Abstract

Bulk milk somatic cell count (BMSCC), individual cow somatic cell count (ICSCC), and incidence rate of clinical mastitis (IRCM) are all udder health parameters. So far, no studies have been reported on the effect of season on BMSCC, IRCM, and ICSCC in the same herds and time period over multiple years. The objectives of this study were to determine the seasonal pattern over a four-year time period of: 1) BMSCC, 2) elevated ICSCC, 3) IRCM, and 4) pathogen-specific IRCM. Bulk milk somatic cell count, ICSCC, and pathogen-specific clinical mastitis data were recorded in 300 Dutch dairy farms. For the analyses of BMSCC, ICSCC, and IRCM a mixed, a transitional, and a discrete time survival analysis model were used, respectively. Sine and cosine were included in the models to investigate seasonal patterns in the data. For all parameters a seasonal effect was present. Bulk milk somatic cell count peaked in August to September in all four years. The probability of cows getting or maintaining a high ICSCC was highest in August and May, respectively. Older and late lactation cows were more likely to develop or maintain a high ICSCC. Incidence rate of clinical mastitis was highest in December to January, except for *Streptococcus uberis* IRCM, which was highest in August. Totally confined herds had a higher *Escherichia coli* IRCM in summer than in winter. Compared with the major mastitis pathogens, the seasonal differences in IRCM were smaller for the minor pathogens. Distinguishing between *Strep. uberis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, and other streptococci is essential when identifying *Streptococcus* spp., because each of them has a unique epidemiology. *Streptococcus uberis* IRCM seems to be associated with being on pasture, whereas *E. coli* IRCM is more housing-related.

6.2 Introduction

Environmental and climatologic factors affect the incidence of many diseases and disorders in dairy cows, such as mastitis (Morse et al., 1988; Whitaker et al., 2004). Therefore, incidence of these diseases often has a seasonal pattern. This seasonal pattern, however, can also be the result of a season-specific average stage of lactation of the herd, especially in herds where the calving pattern of dairy cows tends to be seasonal.

Bulk milk SCC (BMSCC) is mainly influenced by the prevalence of (sub)clinical mastitis in a herd. Prevalence and incidence of subclinical and clinical mastitis depend on factors such as parity, stage of lactation, type of housing and access to pasture, management, and environmental factors, e.g. temperature, humidity, and season (Simensen, 1976; Morse et al., 1988; Hogan and Smith, 1997; Faye et al., 1998). In herds with year-round-calving, SCC had a seasonal pattern, with the highest BMSCC occurring from July to October (Schukken et al., 1993; Sargeant et al., 1998a). Seasonal patterns can also be found in individual cow SCC (ICSCC), with generally the highest ICSCC in July and August (Bodoh et al., 1976; Salsberg et al., 1984). Green et al. (2006) suggested that part of the seasonal variation of BMSCC was caused by the larger proportion of cows with prolonged high ICSCC in the summer. Herds with a seasonal calving pattern in the southern hemisphere, for example in New Zealand, had the highest BMSCC around the calving period in the winter months July to September. The lowest BMSCC in these herds occurred in September to October, shortly after the calving

period, and BMSCC then slowly increased again towards the end of the season in April to May (Clements et al., 2005).

Seasonal effects have also been reported for the incidence rate of clinical mastitis (IRCM), with the highest IRCM for streptococci and coliforms in the summer months June to August in confined U.S. dairy herds (Erskine et al., 1988; Hogan et al., 1989a; Makovec and Ruegg, 2003). Because the epidemiology of each pathogen is unique, the effect on BMSCC and IRCM and its relationship to climatic and environmental factors might be different. Summer humidity and temperature increase coliform counts in bedding material, resulting in an increased coliform IRCM (Smith et al., 1985; Erskine et al., 1988).

Bulk milk SCC, ICSCC, and IRCM are all udder health parameters. Although studies have been conducted to determine the influence of season on BMSCC (Schukken et al., 1992), IRCM (Morse et al., 1988; Hogan et al., 1989a), and subclinical mastitis (Green et al., 2006), so far, no studies have been reported on the effect of season on BMSCC, IRCM, and ICSCC in the same herds and time period over multiple years. Additionally, the epidemiology of mastitis differs among the pathogens involved, and when studying the effect of season on the IRCM, ideally pathogen-specific IRCM should be studied. Therefore, the objectives of this study were to determine in the same herds the seasonal pattern over a four-year time period of: 1) BMSCC, 2) elevated ICSCC, 3) IRCM, and 4) pathogen-specific IRCM.

6.3 Materials and methods

6.3.1 *Herds and sampling*

The data used in the present study were described in detail elsewhere (Barkema et al., 1998). In short, based on mean annual BMSCC, 3 categories were defined: < 150,000, 151,000 - 250,000 and 251,000 - 400,000 cells/mL. For each category 100 dairy herds were selected with at least 10 out of 13 preceding measurements and the last 3 of these within that BMSCC category. Furthermore, only herds that housed cows in free-stall barns during winter, participated in a milk recording program, had an annual quota between 300,000 and 900,000 kg, and had cows of the Holstein-Friesian or Dutch Friesian breed were selected. The Dutch national milk recording system (Nederlands Rundvee Syndicaat, Arnhem, The Netherlands) provided information from milk recordings and BMSCC data. Farmers that participated in the study were asked to collect milk samples from cows with signs of clinical mastitis before treatment during the study period and record severity of signs, treatment and affected quarter. Samples were stored in a freezer on the farm (at approximately –20°C) and collected every 6 to 8 weeks for bacteriological culture. Management data about use of pasture or confinement in the summer were derived from a questionnaire conducted on-farm and described elsewhere (Barkema et al., 1999a).

6.3.2 *Data*

For the analyses of the seasonality of the BMSCC, 11,292 monthly BMSCC measurements on 300 farms from January 5, 1992 to December 5, 1995 were used. Every record contained herd identification, BMSCC and the sampling date.

For the analyses of the ICSCC data, test day recordings of 268 dairy farms between January 1, 1992 and December 31, 1995 were used. In total, 32 herds were excluded from this analysis because the farmers indicated that they sampled fewer than 75% of the clinical cases (18 herds), ceased farming activities (8 herds), or did not have regular ICSCC recordings with intervals of less than 6 weeks (6 herds). Each test day record contained information about herd identification, cow identification, parity, calving date, test day date, kg milk fat, kg protein, kg milk production and ICSCC. Records with test days less than 4 days after calving and records with more than 500 days after calving were removed. Records were removed if the test date of that record was more than 35 or less than 21 days apart from the preceding test day.

The dataset for IRCM analysis contained 274 farms with each record representing one lactation that was full or partial within the study period of that farm. A partial lactation started before and ended within the study period or started within and ended after the study period. Only herds in which the farmers indicated that fewer than 75% of clinical cases were sampled and herds that ceased farming activities were excluded from this analysis. Each farm participated in the study for approximately 18 months between January 1, 1992 and December 31, 1995. Data consisted of herd identification, cow identification, parity, calving date, expected calving date, cull date, dry-off date, on-farm arrival date, date the herd entered the study, date the herd exited the study, and dates and culture results of up to 3 clinical mastitis cases per lactation. Records with biologically impossible combinations of dates were removed, leaving 49,777 full or partial lactations of 29,258 cows for the analyses. At least one recorded case of clinical mastitis occurred in 6,168 lactations. Distribution of pathogens isolated from milk samples from clinical cases was described elsewhere (Barkema et al., 1998).

In short, the major pathogens *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* were isolated 1,501 (21.3% of all cases), 1,666 (23.7%), 946 (13.4%), and 513 (7.3%) times, respectively, the minor pathogens coagulase-negative staphylococci and *Corynebacterium bovis* were isolated 456 (6.5%) and 420 (6.0%) times, respectively, and samples that were culture-negative occurred 1,083 (15.4%) times.

6.3.3 *Statistical analyses*

Seasonality of BMSCC of all 300 herds that started in the study was assessed using a mixed model with herd random effects and auto correlated errors for the repeated measures on herds (PROC MIXED; SAS software version 8.2; SAS Institute, Inc., Cary, NC). To approximate the normal distribution, a natural logarithmic transformation of the BMSCC divided by 1,000 cells/mL was used, which is the optimum transformation for SCC in milk (Ali and Shook, 1980). Sine and cosine terms with a yearly period were included in the model to estimate the seasonal effect (Stolwijk et al., 1999). To correct for year effects, separate parameters were estimated for each year. The model in year k for the j^{th} measurement at herd i was as follows:

$$\ln(\text{BMSCC})_{ijk} = \beta_{0k} + \beta_{1k}\sin(2\pi * \text{day}_{ijk} / 365.25) + \beta_{2k}\cos(2\pi * \text{day}_{ijk} / 365.25) + u_i + \varepsilon_{ijk} \quad (1)$$

where ln(BMSCC) = natural log of BMSCC, β_{0k} = intercept in year k, β_{1k}, β_{2k} = regression coefficients in year k, u_i = random effect for herd i, and ε_{ijk} = residual error.

The within-herd autocorrelation was modeled by a power function with an additional nugget effect (Littell et al., 1996).

Incidence rate of subclinical mastitis was assessed using a 4-level hierarchical transitional model, while including random effects of herds, cows, and lactations. An ICSCC $\geq$ 200,000 cells/mL was considered a high ICSCC and an ICSCC < 200,000 cells/mL a low ICSCC. Two consecutive ICSCC test days were classified in 4 categories: 'low', 2 consecutive low ICSCC test days; 'new', a low ICSCC followed by a high ICSCC; 'chronic', 2 consecutive high ICSCCs, and 'cure', high ICSCC followed by a low ICSCC, as detailed e.g. in Schukken et al. (2003). Parity was evaluated against the proportion of high ICSCC and was categorized in 4 categories: heifers, second and third parity, fourth and fifth parity, and sixth and later parities. The model for the probability of high ICSCC at test day l within the k^{th} lactation of cow j in herd i was as follows:

$$\begin{aligned}
\text{logit}(p_{ijkl}) = {} & \beta_0 + \beta_1 \text{prev_hiscc}_{ijkl} + \beta_2 \text{prev_hiscc}_{ijkl} * \sin(2\pi * \text{day}_{ijkl} / 365.25) \\
& + \beta_3 \text{prev_hiscc}_{ijkl} * \cos(2\pi * \text{day}_{ijkl} / 365.25) \\
& + \beta_4 (1 - \text{prev_hiscc}_{ijkl}) * \sin(2\pi * \text{day}_{ijkl} / 365.25) \\
& + \beta_5 (1 - \text{prev_hiscc}_{ijkl}) * \cos(2\pi * \text{day}_{ijkl} / 365.25) \\
& + \beta_6 \text{dim}_{ijkl} + \beta_7 \text{parity_cat23}_{ijk} + \beta_8 \text{parity_cat45}_{ijk} + \beta_9 \text{parity_cat6}_{ijk} \\
& + \beta_{10} (\text{parity_cat23} * \text{prev_hiscc})_{ijkl} \\
& + \beta_{11} (\text{parity_cat45} * \text{prev_hiscc})_{ijkl} \\
& + \beta_{12} (\text{parity_cat6} * \text{prev_hiscc})_{ijkl} + \beta_{13} \text{yr1992}_{ijkl} + \beta_{14} \text{yr1993}_{ijkl} \\
& + \beta_{15} \text{yr1994}_{ijkl} + \beta_{16} \text{yr1995}_{ijkl} + u_i + v_{ij} + w_{ijk} \qquad (2)
\end{aligned}$$

where p_{ijkl} is the probability of a high ICSCC, β_1 the coefficient for the previous test day being a high ICSCC, β_2 and β_3 the coefficients for the sine and cosine in case the previous ICSCC was high, β_4 and β_5 the coefficients for the sine and cosine in case the previous ICSCC was low, β_6 the coefficient for lactation stage or DIM, β_7 to β_9 coefficients for parity, β_{10} to β_{13} coefficients for the interaction of parity and previous high ICSCC, β_{14} to β_{16} coefficients for years, and u_i, v_{ij} and w_{ijk} the random effects for herd i, cow j, and lactation k, respectively, where i = herd, j = cow within herd, k = lactation within cow, and l = test day within lactation. First order marginal quasi-likelihood estimates of coefficients were derived using the restricted generalized iterative least-squares algorithm in MLwiN (Rasbash et al., 2000). Intra-class correlation coefficients were estimated using the latent variable approximation (Vigre et al., 2004).

Seasonal effects on IRCM were estimated for either all cases or pathogen-specific cases using a multi-level discrete time survival analysis with herd and cow random effects (Singer and Willett, 1993; Singer and Willett, 2003). To be able to use calendar time as a predictor in the analysis, biological time, DIM, was used as the survival time. Days in milk were categorized in periods of 14 days. Lactation periods after 420 DIM (30 periods of 14 days) were omitted, because pathogen-specific IRCM after that time was low or zero per time period. A second or third case of clinical mastitis in the same lactation, regardless of culture result, was considered a new case if there were at least 14 days between the previous and the current case of clinical mastitis. The first consecutive 14-day period at risk in a lactation that started at least 14 days after a case of clinical mastitis was included in the analysis again. Left truncated lactations were considered at risk from the first complete 14-day period starting after the day the

herd entered the study. Lactations were right censored after the last full 14-day period before the end of the study period. Variables for year were included in the model; however, herds participated in the study for no longer than 1.5 yr. Therefore, the random effect of herds will account for a large part of the year effect. Year effect did not change the coefficients of interest substantially. For simplicity, the variable for year effect was omitted from the model. For all clinical mastitis cases and for all pathogen-specific cases, a 3-category variable containing summer housing data (outside day and night, inside at night, and inside day and night), was included in the model including its interactions with the season variables. These variables were removed if they were not significant. The basic model for the hazard of a 'failure' (clinical mastitis) in period k of cow j in herd i was as follows:

$$\begin{aligned}\text{logit}(p_{ijk}) = {} & \alpha_k + \beta_1 \sin(2\pi * \text{day}_{ijk} / 365.25) + \beta_2 \cos(2\pi * \text{day}_{ijk} / 365.25) \\ & + \beta_3 \text{parity\_cat23}_{ij} + \beta_4 \text{parity\_cat45}_{ij} + \beta_5 \text{parity\_cat6}_{ij} \\ & + \beta_6 \text{night\_in}_i + \beta_7 \text{confined}_i + u_i + v_{ij} \end{aligned} \quad (3)$$

where p_{ijk} is the hazard of a 'failure' (clinical mastitis), and on logistic scale, α_k is the baseline hazard for the k^{th} 14-day period in the lactation, β_1 and β_2 are the coefficients for the sine and cosine terms, β_3 for second and third parities, β_4 for fourth and fifth parities, β_5 for sixth and later parities, β_6 for herds that keep their cows inside at night, β_7 for totally confined herds, and u_i and v_{ij} the random effects for herd i and cow j (within herd i), respectively. First order marginal quasi-likelihood estimates of coefficients were derived using the restricted generalized iterative least-squares algorithm in MLwiN (Rasbash et al., 2000). Intra-class correlation coefficients were estimated using the

latent variable approximation (Vigre et al., 2004). Model (3) was also used for pathogen-specific IRCM where p_{ijk} is the hazard of a case of clinical mastitis caused by a specific pathogen. Amplitude α and phase shift φ of the predicted sine waves in equations (1), (2), and (3) were calculated using formulas that were described earlier (Stolwijk et al., 1999). Standard errors of the amplitude and phase shift were approximated using the 'delta method' (Weisberg, 2005). Formulas are available from the authors on request. Winter, spring, summer and autumn were defined as the period in a year from December 21 to March 20, March 21 to June 20, June 21 to September 20, and September 21 to December 20, respectively.

6.4 Results

The number of cows that calved per week changed over the year, both for heifers and for multiparous cows. More cows and heifers calved in autumn and fewer calved in summer (Fig. 1). This difference was larger for heifers than for cows. Of 9,293 heifers that calved during the study period, 324 calved in June compared with 1,169 in September, while out of 22,620 multiparous cows that calved during the study period 1,663 calved in June compared with 2,057 in September (Fig. 1).

6.4.1 *Bulk Milk SCC*

Bulk milk SCC of the 300 farms ranged from 28,000 to 740,000 cells/mL with a geometric mean of 187,000 cells/mL across all farms over the study period.

Season had a significant effect on geometric mean BMSCC per day of the study period in all 4 yr (Fig. 2; Table 1). The amplitude of the seasonal effect differed among

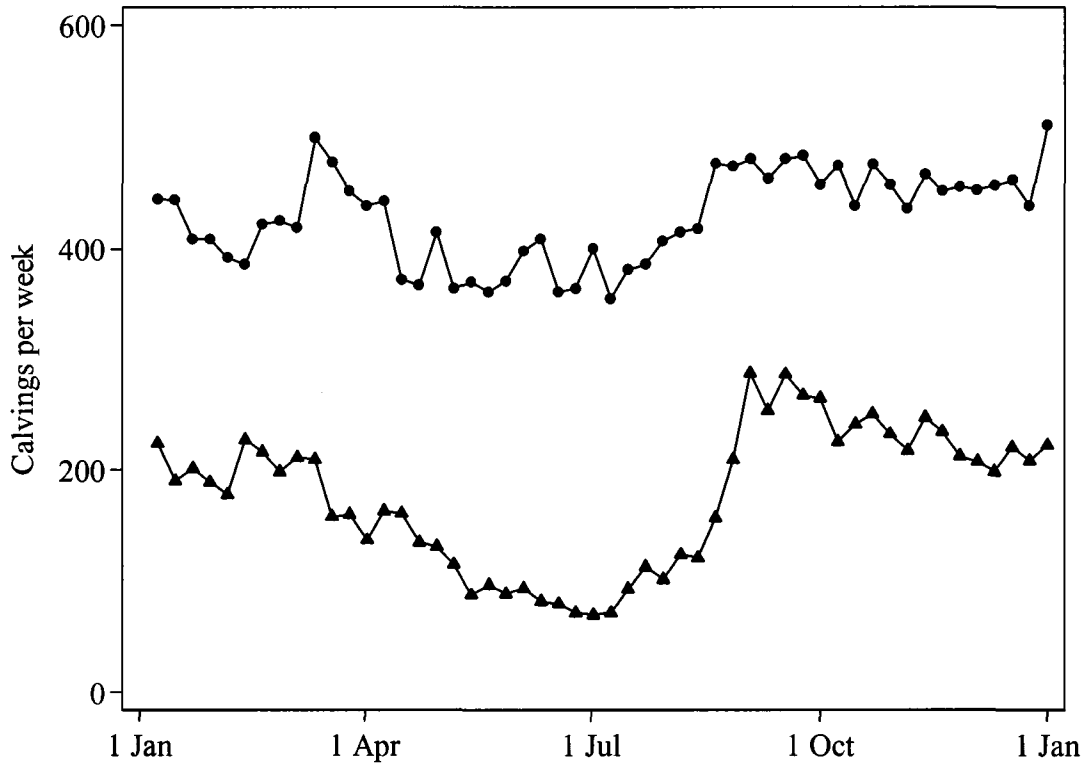

Figure 1. Average weekly number of calvings during the study period for heifers (▲) and multiparous cows (●) in 300 Dutch dairy herds.

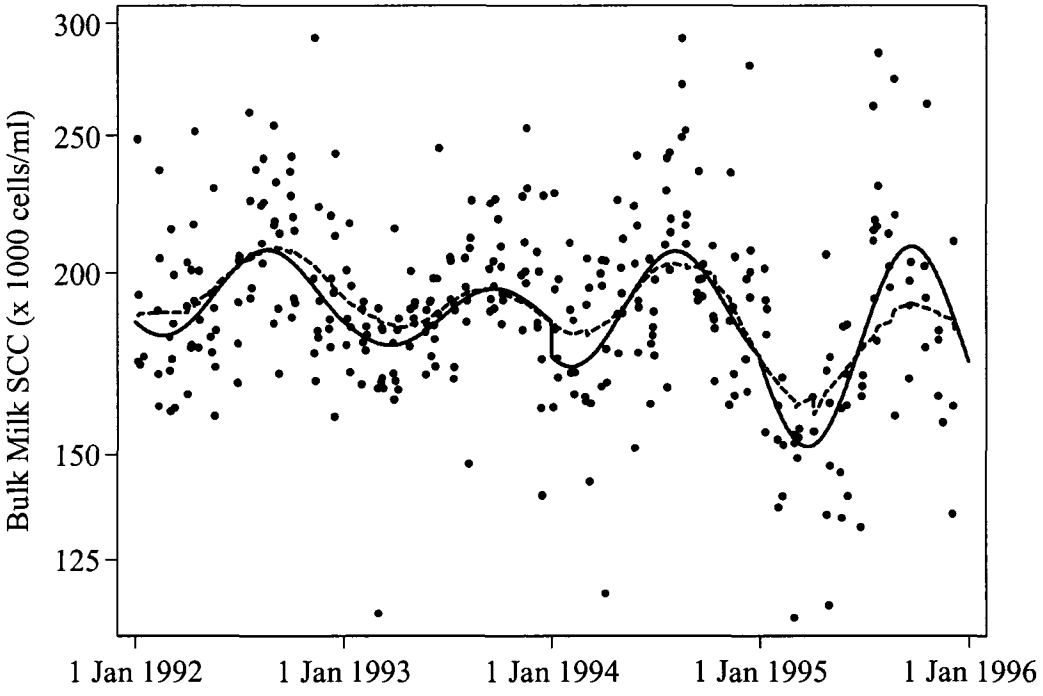

Figure 2. Geometric mean weekly bulk milk SCC of 300 Dutch dairy herds from January 1992 to January 1996, lowess smoother (dashed line, bandwidth 0.2), and model prediction (solid line).

Table 1. Final model of seasonal variation of the natural log of bulk milk SCC (1,000 cells/mL) of 300 Dutch dairy herds.

	1992	1993	1994	1995
Intercept (SE)	5.27 (0.024)	5.23 (0.023)	5.24 (0.023)	5.18 (0.028)
Sine (SE)	-0.052 (0.012)	-0.046 (0.010)	-0.053 (0.010)	-0.162 (0.018)
Cosine (SE)	-0.046 (0.010)	-0.007 (0.008)	-0.078 (0.009)	-0.023 (0.015)
Amplitude α (SE)	0.070 (0.011)	0.046 (0.010)	0.094 (0.009)	0.164 (0.018)
Phase shift φ (SE)	0.729 (0.156)	0.144 (0.181)	0.973 (0.103)	0.143 (0.093)
Predicted peak date	Aug. 18	Sep. 21	Aug. 4	Sep. 21
95% Confidence interval	Jul. 31 – Sep. 4	Aug. 31 – Oct. 12	Jul. 23 – Aug. 16	Sep. 11 – Oct. 2
Variance parameters				
Between herd variance σ_h^2 (SE)		0.126 (0.012)		
Within-herd error variance σ^2 (SE)		0.068 (0.003)		
Within-herd correlation[1] ρ (SE)		0.993 (0.001)		
Within-herd nugget effect[2] σ_1^2 (SE)		0.046 (0.001)		

[1]Between outcomes at two test days d days apart, the correlation is $[\sigma_h^2 + \sigma^2\rho^d] / [\sigma_h^2 + \sigma^2 + \sigma_1^2]$.
[2]See Littell et al. (1996).

the 4 yr included in the study and was largest in 1995 (Table 1; page 164). Modeled BMSCC was highest at 209,000 cells/mL in September 1995 and lowest at 150,000 cells/mL in March 1995. The seasonal variation of geometric mean BMSCC within a year ranged between 26,000 cells/mL in 1993 and 59,000 cells/mL in 1995. There were no differences in geometric mean BMSCC between the 3 categories of summer housing: outside day and night, inside at night, and inside day and night ($P >$ 0.5).

6.4.2 *High ICSCC*

Data structure of ICSCC records that were used in the transitional model consisted of 4 levels: herd (268), cow within herd (31,007), lactation within cow (59,200), test day within lactation (409,932). Of 409,932 test day recordings in the final dataset for ICSCC, 23.8% were ≥ 200,000 cells/mL: heifers had 11.8% high ICSCC test day recordings out of 127,968, while 29.3% of the records of the multiparous cows had a high ICSCC. In the transitional model, predictors for season were significant, meaning that both patterns of 'new' high ICSCC and 'chronic' high ICSCC were seasonal (Table 2; Fig. 3 and 4). Because the 12-month sine wave in the model can only show 1 peak, it puts the peak of 'new' cases of high ICSCC in the summer on August 7 (95% confidence interval: Jul. 26 – Aug. 19), and therefore the dip in February. Although accounted for, the model could not show a second, shorter lasting peak in 'new' high ICSCC for both heifers and multiparous cows in May (Table 2, Fig. 3). 'Chronic' high ICSCC cases more often occurred in spring with a predicted peak on Apr. 28 (95% confidence interval: Apr. 13 - May 12), which is caused by a larger proportion of 'chronic' high ICSCC in May to September in heifers and a peak in April and May in

Table 2. Final model for the odds of ICSCC ≥ 200,000 cells/mL ('high' ICSCC) on the logit scale.

Variable	Variance (SE)	% Variance
Random effects		
Herd	0.113 (0.011)	2.9
Cow	0.511 (0.011)	12.9
Lactation	0.038 (0.009)	1.0
Test day1	-	83.2
	β (SE)	P
Fixed effects		
Intercept	-3.421 (0.035)	< 0.001
Previous high ICSCC	2.254 (0.024)	< 0.001
Parity		< 0.001
Heifers	0 (Ref.)	
2-3rd parity	0.761 (0.016)	
4-5th parity	1.301 (0.020)	
≥ 6th parity	1.663 (0.025)	
Parity x Previous high ICSCC		< 0.001
Heifers	0 (Ref.)	
2-3rd parity	-0.120 (0.028)	
4-5th parity	-0.175 (0.031)	
≥ 6th parity	-0.121 (0.036)	
Days in milk (x 100)	-0.369 (0.005)	< 0.001
Season 'new' high ICSCC2		< 0.001
Sine	-0.046 (0.008)	
Cosine	-0.061 (0.008)	
Season 'chronic' high ICSCC3		< 0.001
Sine	0.077 (0.011)	
Cosine	-0.039 (0.011)	
Year		< 0.001
1992	0 (Ref.)	
1993	0.060 (0.025)	
1994	0.061 (0.025)	
1995	-0.076 (0.026)	

[1]Assumes level 1 variance on the logit scale is $\pi^2 / 3$, where $\pi = 3.1416...$ (Vigre et al., 2004).

[2] 'New' high ICSCC = previous low (< 200,000 cells/mL), current high ICSCC.

[3] 'Chronic' high ICSCC = previous high and current high ICSCC.

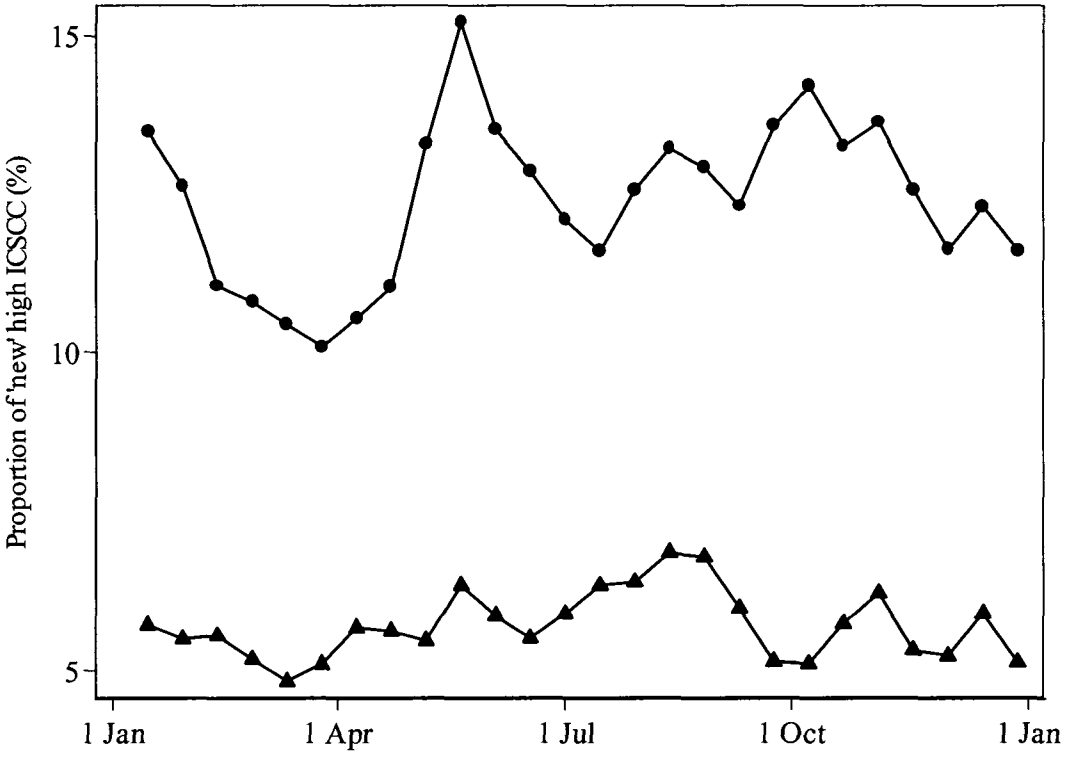

Figure 3. Proportion of 'new' high ICSCC (ICSCC ≥ 200,000 cells/mL) per 14-day period for heifers (▲) or multiparous cows (●) that had low ICSCC at the previous test day.

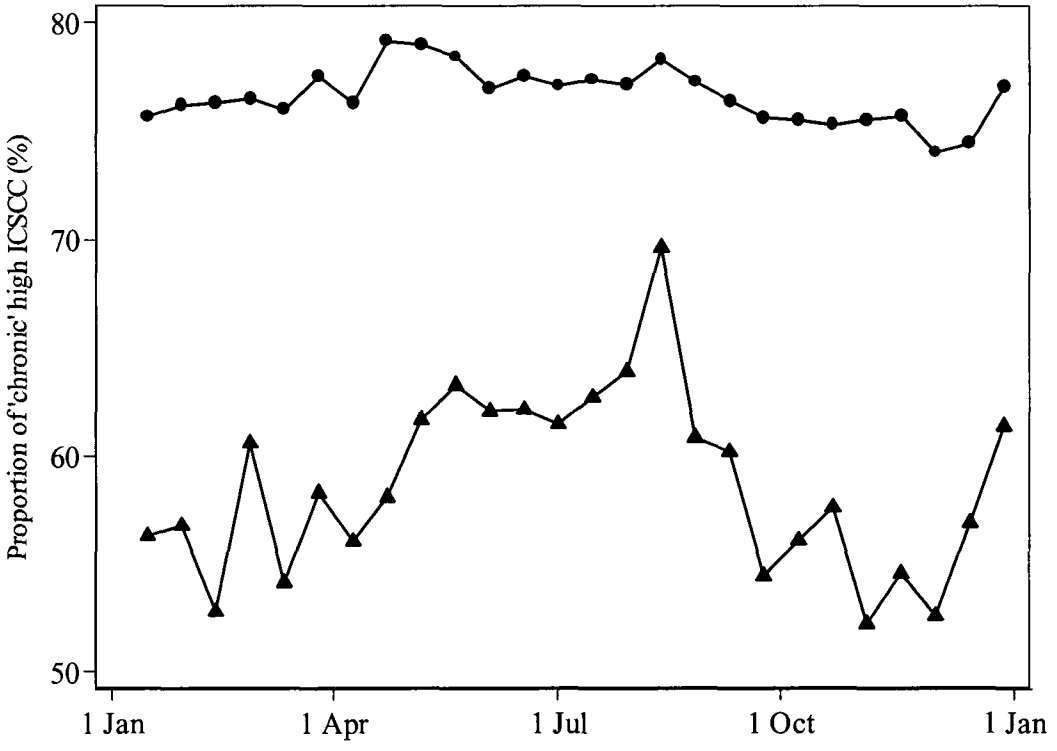

Figure 4. Proportion of 'chronic' high ICSCC (ICSCC ≥ 200,000 cells/mL) per 14-day period for heifers (▲) or multiparous cows (●) that had high ICSCC at the previous test day.

multiparous cows (Table 2; page 166; Fig. 4; page 168). Older cows more often developed and maintained high ICSCC than younger cows (Table 2; Fig. 3 and 4; pages 166 to 168). Cows that were further in lactation more often had a high ICSCC than cows early in lactation (Table 2; page 166). Proportions of variance explained at herd, cow, lactation, and test day level were 2.9, 12.9, 1.0, and 83.2%, respectively (Table 2; page 166).

6.4.3 *Incidence Rate of Clinical Mastitis*

In total, 7,083 cases of clinical mastitis were analyzed in the final dataset. Data structure consisted of 3 levels: herd (274), cow within herd (29,258), and lactation within cow (49,777). The proportion of variance explained at cow-level was larger for pathogen-specific cases of clinical mastitis than for all cases of clinical mastitis, the largest being for *Strep. uberis* IRCM, closely followed by *C. bovis* IRCM (Table 3). Proportion of variance that was explained at the herd-level was largest for culture-negative IRCM followed by *Staph. aureus* IRCM. Proportion of variance at herd-level was the largest for culture negative clinical mastitis, followed at some distance by *Staph. aureus* and *E. coli* IRCM, respectively (Table 3).

The IRCM was highest in the first 14-day period after calving, declined steeply in the second period and then, after a rise for multiparous cows only, declined slower over the rest of the lactation (Fig. 5). This second peak was most pronounced for *Staph. aureus* and *E. coli* IRCM, whereas no second lactational peak could be found for *Strep. uberis* IRCM (Fig. 6). Also, compared with heifers, multiparous cows were more likely to get clinical mastitis, and had a higher IRCM over the whole lactation for all pathogens (Table 3). The IRCM increased with increasing parity (Table 3).

Table 3. Final model estimates of coefficients and variances of general and pathogen-specific incidence rate of clinical mastitis on 274 Dutch dairy farms on the logit scale.

Variables	All cases		*Escherichia coli*		*Staphylococcus aureus*		*Streptococcus dysgalactiae*	
	Var.[4] (SE)	% var.	Var. (SE)	% var.	Var. (SE)	% var.	Var. (SE)	% var.
Random effects								
Herd	0.313 (0.031)	7.1	0.510 (0.062)	9.3	0.673 (0.075)	10.1	0.477 (0.069)	7.6
Cow	0.785 (0.037)	17.9	1.657 (0.152)	30.4	2.678 (0.149)	40.3	2.508 (0.241)	40.0
Lactation[1]	-	75.0	-	60.3	-	49.5	-	52.4
Fixed effects	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P
Season		< 0.001		< 0.001		< 0.001		< 0.001
Sine	-0.051 (0.017)		0.006 (0.053)		0.134 (0.035)		0.198 (0.048)	
Cosine	0.194 (0.017)		0.412 (0.054)		0.215 (0.036)		0.563 (0.049)	
Parity		< 0.001		< 0.001		< 0.001		< 0.001
1st parity	0 (Ref.)		0 (Ref.)		0 (Ref.)		0 (Ref.)	
2-3rd parity	0.560 (0.036)		0.811 (0.081)		0.618 (0.075)		0.314 (0.092)	
4-5th parity	0.852 (0.040)		1.134 (0.088)		0.909 (0.082)		0.643 (0.101)	
≥ 6th parity	1.046 (0.045)		1.222 (0.099)		1.252 (0.090)		1.034 (0.109)	
Housing[2]		-[3]		0.04		-		-
Outside all day	-		0 (Ref.)		-		-	
Inside at night	-		0.245 (0.107)		-		-	
Totally confined	-		0.413 (0.264)				-	
Season x Housing		-		< 0.001		-		-
Sine * Inside at night	-		-0.135 (0.077)		-		-	
Cosine * Inside at night	-		-0.338 (0.078)		-		-	
Sine * Totally confined	-		0.064 (0.183)		-		-	
Cosine * Totally confined	-		-0.595 (0.189)		-		-	

[1]Assumes level 1 variance on the logit scale is $\pi^2 / 3$, where $\pi = 3.1416\ldots$ (Vigre et al., 2004).
[2]Housing during summer; all cows were confined during winter.
[3]Non-significant effects ($P > 0.05$) were removed from the model.
[4]Var. = Variance

Table 3. (*Continued*).

Variables	*Streptococcus uberis*		Coagulase-negative staphylococci		*Corynebacterium bovis*		Culture-negative	
	Var.[4] (SE)	% var.	Var. (SE)	% var.	Var. (SE)	% var.	Var. (SE)	% var.
Random effects								
Herd	0.520 (0.096)	6.6	0.480 (0.095)	7.3	0.598 (0.112)	7.6	0.985 (0.108)	16.7
Cow	4.085 (0.415)	51.7	2.849 (0.489)	43.0	3.955 (0.511)	50.4	1.617 (0.207)	27.4
Lactation[1]	-	41.7	-	49.7	-	41.9	-	55.8
Fixed effects	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
Season		< 0.001		0.03		0.07		< 0.001
Sine	-0.631 (0.069)		0.069 (0.067)		-0.029 (0.070)		-0.013 (0.044)	
Cosine	-0.603 (0.070)		0.162 (0.067)		0.160 (0.070)		0.171 (0.044)	
Parity		< 0.001		0.002		< 0.001		< 0.001
1st parity	0 (Ref.)		0 (Ref.)		0 (Ref.)		0 (Ref.)	
2-3rd parity	0.663 (0.142)		0.169 (0.122)		0.781 (0.157)		0.591 (0.084)	
4-5th parity	1.071 (0.151)		0.390 (0.139)		1.262 (0.164)		0.747 (0.095)	
≥ 6th parity	1.576 (0.157)		0.540 (0.159)		1.345 (0.183)		0.447 (0.121)	
Housing[2]		-[3]		-		-		-
Outside all day	-		-		-		-	
Inside at night	-		-		-		-	
Totally confined	-						-	
Season x Housing		-		-		-		-
Sine * Inside at night	-		-		-		-	
Cosine * Inside at night	-		-		-		-	
Sine * Totally confined	-		-		-		-	
Cosine * Totally confined	-		-		-		-	

[1]Assumes level 1 variance on the logit scale is $\pi^2 / 3$, where $\pi = 3.1416\ldots$ (Vigre et al., 2004).
[2]Housing during summer; all cows were confined during winter.
[3]Non-significant effects ($P > 0.05$) were removed from the model.
[4]Var. = Variance

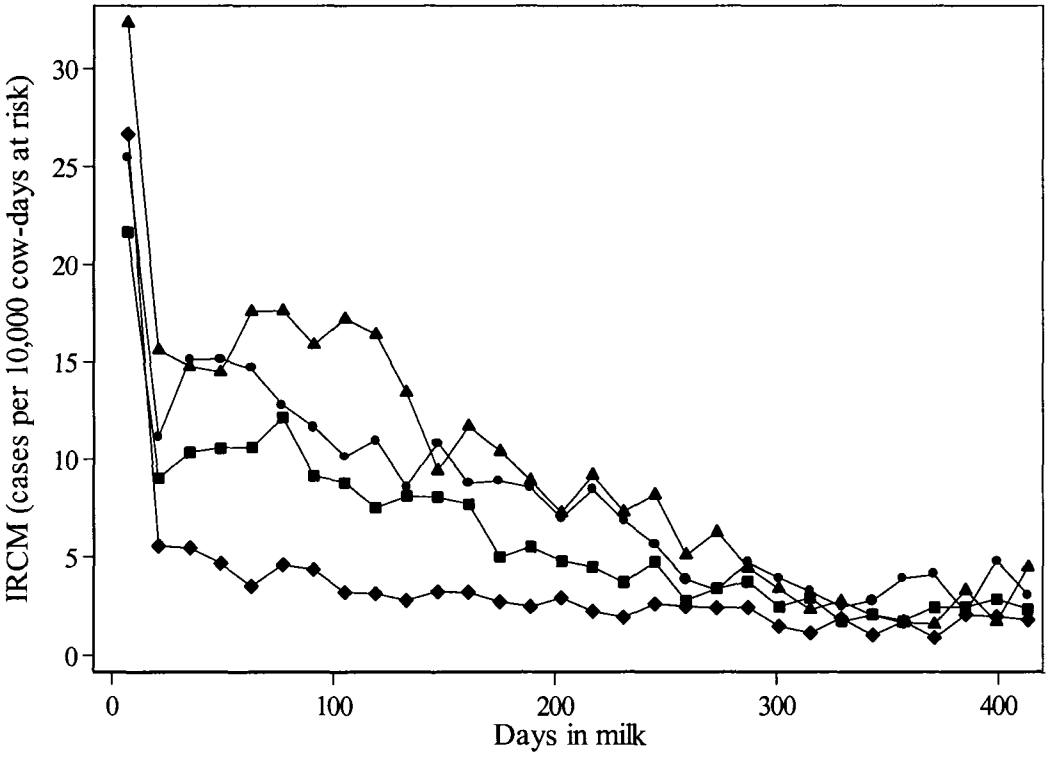

Figure 5. Distribution of incidence rate of clinical mastitis (IRCM) over lactation, for heifers (♦), second and third parity (■), fourth and fifth parity (●), and sixth and later parity cows (▲).

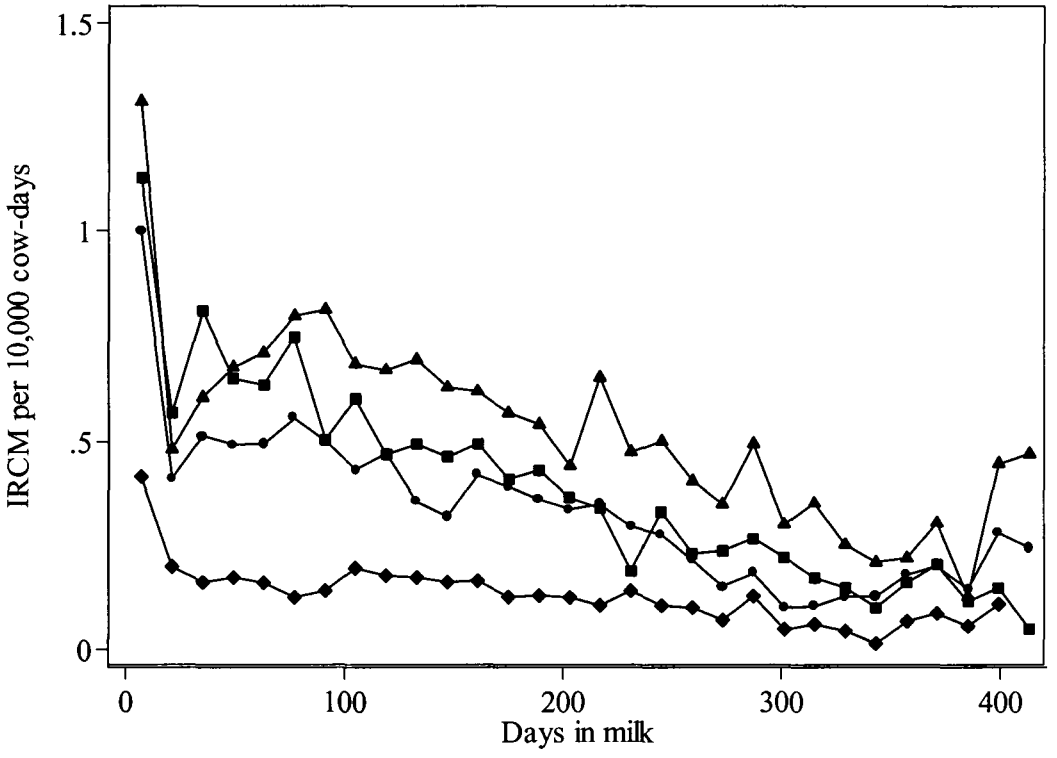

Figure 6. Predicted incidence rates of pathogen-specific clinical mastitis (IRCM) over lactation for heifers. Legend: *Escherichia coli* (■), *Staphylococcus aureus* (▲), *Streptococcus dysgalactiae* (●), and *Streptococcus uberis* (♦).

An apparent effect of season ($P < 0.001$) was present for IRCM in general (Table 3; page 170). On average, cows were more likely to experience clinical mastitis in late fall (December) than in the summer (Fig. 7; Table 4). A small peak of IRCM appeared in the second half of July, mainly in high BMSCC herds, which is the result of a peak in *Staph. aureus* and *E. coli* IRCM (Fig. 7 and 8). The peak in the high BMSCC category was mainly caused by a peak in *Staph. aureus* and *E. coli* IRCM. A peak in *E. coli* IRCM was also noticed in the medium BMSCC category. For a mid-lactation, second parity cow, IRCM was 6.3 cases per 10,000 cow-days at risk in June and 9.4 cases per 10,000 cow-days at risk in December. Effect of season was also clearly present for most pathogen-specific IRCM ($P < 0.001$), except for *C. bovis* IRCM ($P = 0.07$; Table 3; page 170). *Streptococcus uberis* IRCM was highest in the summer (August), dependent on summer housing strategy for *E. coli*, and highest in December and January for other pathogens (Fig. 8 and 9; Table 3; page 170; and Table 4). Seasonal differences were largest for *Strep. uberis*, followed by *Strep. dysgalactiae* and smallest for *E. coli* in herds that kept cows inside only at night during the summer (Table 4).

All farms kept their cows inside during the winter months. During summer, lactating cows were kept inside day and night on 13 (4.5%) farms. On 171 (57%) farms the cows were kept outside day and night, and on the remaining 116 (39%) farms the cows were kept inside at night only. The interaction of housing strategy with the season variables was only significant for *E. coli* IRCM. Cows that were confined in the summer were more likely to develop clinical *E. coli* mastitis in the summer than in the winter, while cows that were on pasture day and night during the summer had a higher *E. coli* IRCM in the winter (Table 3; page 170 and Table 4). *Streptococcus uberis* IRCM was numerically lower in summer in totally confined herds and had no seasonal

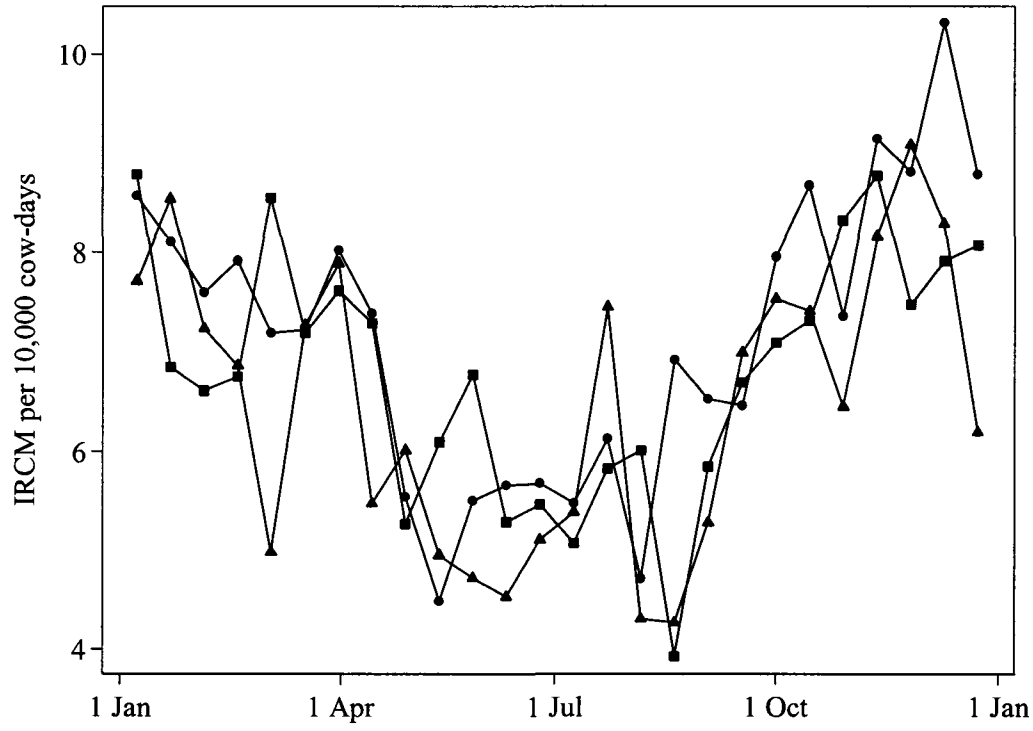

Figure 7. Incidence rate of clinical mastitis (IRCM) per 14-day period for herds with low (< 150,000 cells/mL) (●), medium (150,000 – 250,000 cells/mL) (■), and high (250,000-400,000 cells/mL) BMSCC (▲).

Table 4. Derived parameters from the final model estimates of coefficients and variances of general and pathogen-specific incidence rate of clinical mastitis on 274 Dutch dairy herds (Stolwijk et al., 1999; Weisberg, 2005). Formulas are available on request from the authors.

Pathogen	Amplitude α (SE)	Phase shift φ (SE)	Peak day	95% CI[1]
All cases	0.201 (0.017)	1.828 (0.086)	Dec. 15	Dec. 5 – Dec. 25
Escherichia coli				
Outside	0.412 (0.054)	1.556 (0.129)	Dec. 31	Dec. 16 – Jan 14
Inside at night only	0.149 (0.056)	2.615 (0.375)	Oct. 30	Sep. 17 – Dec. 12
Totally confined	0.195 (0.180)	-1.204 (0.900)	Jun. 9	Feb. 26 – Sep. 20
Staphylococcus aureus	0.253 (0.036)	1.013 (0.140)	Jan. 31	Jan. 15 – Feb. 16
Streptococcus dysgalactiae	0.597 (0.049)	1.233 (0.080)	Jan. 18	Jan. 9 – Jan. 27
Streptococcus uberis	0.873 (0.073)	3.905 (0.076)	Aug. 16	Aug. 7 – Aug. 25
Coagulase-negative staphylococci	0.176 (0.067)	1.168 (0.380)	Jan. 22	Dec. 10 – Mar. 6
Corynebacterium bovis	0.163 (0.070)	1.750 (0.429)	Dec. 19	Oct. 31 – Feb. 6
Culture-negative	0.172 (0.044)	1.647 (0.254)	Dec. 25	Nov. 26 – Jan. 23

[1]CI = Confidence interval

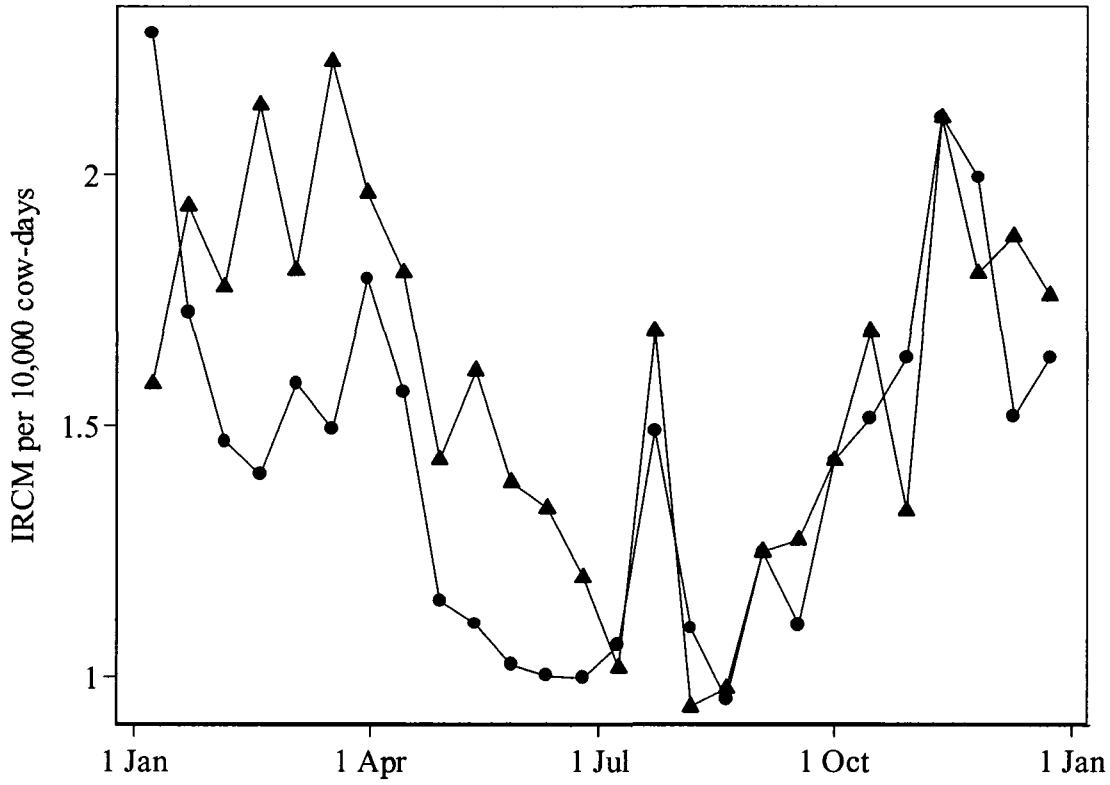

Figure 8. *Staphylococcus aureus* (●) and *Escherichia coli* (▲) incidence rate of clinical mastitis (IRCM) per 14-day period.

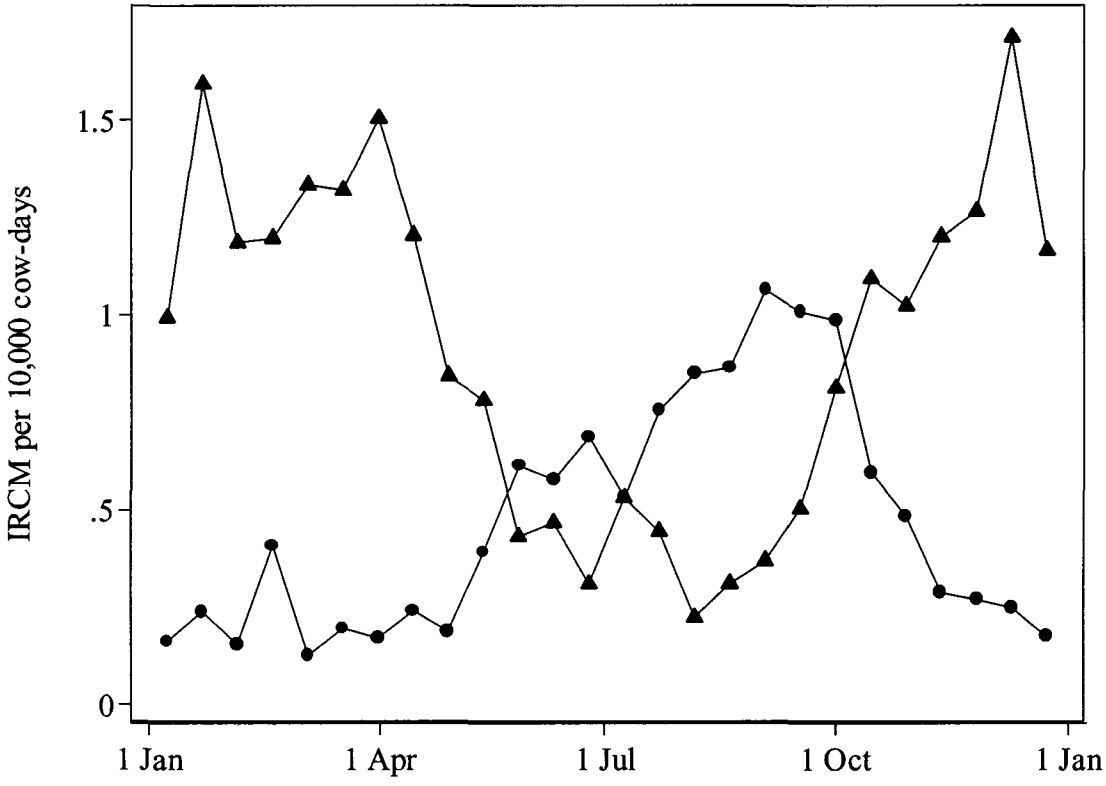

Figure 9. *Streptococcus uberis* (●) and *Streptococcus dysgalactiae* (▲) incidence rate of clinical mastitis (IRCM) per 14-day period.

effect ($P = 0.14$). For example, on August 1, for a mid-lactation, second parity cow, *Strep. uberis* IRCM was 1.1 cases per 10,000 cow-days at risk on pasture and 0.3 cases per 10,000 cow-days at risk in a totally confined herd.

6.5 Discussion

A pronounced association was found between season and udder health parameters BMSCC, IRCM, pathogen-specific IRCM, and ICSCC corrected for stage of lactation. Predicted BMSCC and predicted 'new' high ICSCC peaked in August to September, whereas the peak for most pathogen-specific IRCM was in December or January. *Streptococcus uberis* IRCM and *E. coli* IRCM in semi and total confined herds peaked in August, October, and June, respectively.

Calving was not evenly distributed over the year, and stage of lactation and parity are associated with ICSCC (Dohoo et al., 1984; Laevens et al., 1997; Green et al., 2004). Therefore, these variables were added to the models for IRCM and ICSCC. Not correcting for these variables would result in a bias in the effect of season on ICSCC and IRCM around the calving periods. Discrete time survival analyses for IRCM allowed to model calendar time as continuous predictor using a sine function, while biological time, or days in milk, could be modeled in 14-day periods. A drawback of using 14-day periods is that some data was lost by excluding periods at risk which were shorter than 14 days. Using shorter periods, however, would require more computer power than was available at the time of analysis. For the same reason continuous time survival analyses for this dataset would be less feasible if multi-level random effects and time varying covariates were to be added to the model.

The seasonal variation of BMSCC is in agreement with other studies which used a similar model to predict BMSCC (Sargeant et al., 1998a; Norman et al., 2000). In those studies, BMSCC peaked in late summer or fall as well. Because BMSCC is the product of the ICSCC and milk production of the cows that are included in the bulk tank, it is logical that the prevalence of high ICSCC follows the same pattern as the BMSCC. In a study conducted in 33 British dairy herds, Green et al. (2006) suggested that the increase in BMSCC in this period is the result of an increase in chronic high ICSCC. By contrast, in our study the incidence of 'new' elevated ICSCC was highest in August with a shorter lasting peak in April (Fig. 3), and the peak in August coincided with the peak of the BMSCC (Fig. 2), whereas the largest proportions of 'chronic' high ICSCC were found in April (Fig. 4). In Norway, prevalence of *Staph. aureus* and *Strep. uberis* IMI was highest in spring and summer, respectively (Østerås et al., 2006). *Staphylococcus aureus* and *Strep. uberis* IMI can cause clinical and subclinical mastitis with a prolonged period of high ICSCC (De Haas et al., 2004). Therefore, a possible explanation for the predicted rise in probability of becoming a 'chronic' or a 'new' high ICSCC cow in April is an increased incidence of subclinical IMI caused by *Staph. aureus,* whereas the rise in 'new' high ICSCC in August can be explained by an increased incidence of *Strep. uberis* IMI in that period. Another explanation could be that on farms that had difficulties producing the annual milk quota, high SCC cows that should be culled were kept longer on farm till May, the start of the new 'quota year'.

Staphylococcus aureus, E. coli, and *Strep. dysgalactiae* IRCM peaked in December and January, while *Strep. uberis* IRCM was highest in August. Seasonal fluctuation of coagulase-negative staphylococci and *C. bovis* IRCM were less pronounced. The peak in winter for most major pathogens was not in agreement with

other studies (Smith et al., 1985; Erskine et al., 1988; Hogan et al., 1989b; Todhunter et al., 1991; Makovec and Ruegg, 2003), which reported a peak in summer for both coliforms and streptococci. These studies, however, were all performed in confined US herds. In our study, in totally confined herds *E. coli* IRCM was also higher in the summer compared with the pastured herds, which had the *E. coli* IRCM peak in winter, while *Escherichia coli* IRCM was lower in summer in herds that kept their cows on pasture day and night (Barkema et al., 1999b). In totally confined herds, the summer heat and humidity of the cows' environment enhance the growth of *E. coli* in the environment, resulting in high coliform counts in bedding (Smith et al., 1985; Hogan et al., 1989b; Goldberg et al., 1992), and therefore a greater exposure to this pathogen.

The epidemiology of particularly *Strep. uberis* IMI and mastitis are not well understood. In this study, *Strep. uberis* IRCM peaked in summer. In Norway prevalence of *Strep. uberis* IMI peaked in summer (Østerås et al., 2006). *Streptococcus uberis* IRCM was numerically lower in totally confined herds in summer than in herds that pastured the cows (results not shown). In a recent study by Zadoks et al. (2005), the proportion of fecal samples containing *Strep. uberis* was larger during the summer grazing season than during winter. In New Zealand, where cows are pastured the whole year, *Strep. uberis* is the most important mastitis pathogen, whereas clinical *E. coli* mastitis is relatively uncommon (Pankey et al., 1996; McDougall, 1998). Zadoks et al. (2005) could not find *Strep. uberis* in haylage, but found it in soil samples. This indicates that cows on pasture may maintain a contamination cycle through the feces. As a results, the infection pressure for *Strep. uberis* increases on pasture. A role of pasture contamination in the epidemiology of *Strep. uberis* has also been suggested by Cullen and Little (1969). In summary, evidence is mounting that *Strep. uberis* is a

pasture-associated pathogen, at least in some geographic areas. *Streptococcus uberis* IRCM was high shortly after calving, but did not decline as much as for the other pathogens, and was more or less constant throughout lactation (Fig. 6). This indicates that, contrary to what was found in a confined US dairy herds for *Strep. uberis* IMI (Todhunter et al., 1995), in Dutch dairy herds clinical mastitis caused by *Strep. uberis* more often occurs during lactation, as was also found before by Zadoks et al. (2003). Unlike for *E. coli*, immunosuppression during peak lactation does not seem to play an important role in the pathogenesis of *Strep. uberis* mastitis. The diet, however, could play a role in both *E. coli* and *Strep. uberis* IRCM. When lactating cows in the herd were fed corn silage, a lower overall IRCM and IRCM caused by *Strep. uberis*, and a higher IRCM caused by *E. coli* were observed (Barkema et al., 1999b). Corn silage and haylage are more commonly fed in the winter when cows are kept inside.

A peak was noted in both *E. coli* and *Staph. aureus* IRCM in the second half of July in the high BMSCC category herds. These two pathogens have a different epidemiology and such a peak was not found for other pathogens. The number of cows at risk per day in the raw data in the summer period was approximately the same in this period as in others, whereas the absolute number of clinical mastitis cases per day was somewhat larger in the month July. A possible explanation of this peak could be a flare-up of existing *Staph. aureus* infections in the high BMSCC category herds and new *E. coli* infections in other herds (Fig. 7). Although The Netherlands has a moderate climate, immunosuppression as a result of heat stress may also have played a role. The peak, however, did not have a large effect on the outcome of the *E. coli* and *Staph. aureus* IRCM models.

Herd-level variance for culture-negative samples was larger than for any of the pathogens studied. There are numerous reasons for a milk sample of a clinical mastitis case to be culture-negative. One possibility for this herd-level variation is that some herds had more *Mycoplasma* than others. This seems unlikely, however, because prevalence of *Mycoplasma* mastitis is generally not so high that it could explain most of the culture-negative samples and the clinical appearance of the culture-negative mastitis cases did not indicate *Mycoplasma* mastitis. Milk samples in this study were not tested for *Mycoplasma* spp. because it requires special growth media. Culture-negative results are often attributed to either *E. coli* (Smith and Hogan, 1993) or *Staph. aureus* (Sears et al., 1990). *Staphylococcus aureus* IMI also frequently results in culture-negative milk samples, and certain strains more often result in culture-negative samples than others (Sears et al., 1990). Variation of culture-negative IRCM, however, was in our model not in accordance with herd-level variance of either *Staph. aureus* IRCM nor *E. coli* IRCM and the effect of parity was different for culture-negative on one side and *E. coli* and *Staph. aureus* IRCM on the other side (Table 3). Therefore, samples can be culture-negative for a variety of reasons and might not be representative for one type of bacteria.

Coagulase-negative staphylococci IRCM, and to a lesser extent *Strep. dysgalactiae* IRCM, did not increase as much with parity as IRCM of other pathogens. Because *Strep. dysgalactiae* is mainly a contagious pathogen, and because *Strep. dysgalactiae* has a more favorable response to antimicrobial treatment compared with *Staph. aureus*, prevalence of chronic *Strep. dysgalactiae* mastitis and therefore incidence of clinical flare-ups might be lower. Coagulase-negative staphylococci IRCM might be lower in later parities for a similar reason, because they are a very diverse group of bacteria and some of them might not become chronically infected.

In many North-American studies the group of non-*agalactiae* streptococci was not differentiated (Oliver, 1998; Sargeant et al., 1998b; Makovec and Ruegg, 2003; Gröhn et al., 2004). The results of this study, however, indicate that the epidemiology of two mastitis pathogens in this group which are isolated most frequently, *Strep. dysgalactiae* and *Strep. uberis*, is quite different. This is supported by other studies in terms of herd-level risk factors (Barkema et al., 1999b), response to treatment (Swinkels et al., 2005) and contagiousness (Neave et al., 1969). Additionally, epidemiological characteristics even differ among strains within species (Zadoks et al., 2003). Therefore, in research and also routine bacteriological culture, besides *Strep. agalactiae,* at least *Strep. dysgalactiae* and *Strep. uberis* should be differentiated, whereas in research projects strain typing needs to be considered.

6.6 Conclusion

Season is associated with BMSCC, IRCM, pathogen-specific IRCM, and ICSCC. The increase of BMSCC in August and September cannot fully be explained by IRCM, but is most likely associated with the increase of cows with new high ICSCC and longer periods of high ICSCC. Distinguishing between *Strep. uberis*, *Strep. dysgalactiae*, *Strep. agalactiae*, and other streptococci is essential when identifying *Streptococcus* spp., because each of them has a unique epidemiology. *Streptococcus uberis* IRCM seems to be related to pasture, whereas other streptococci and *E. coli* seems to be more housing-related. Thus, the present study demonstrates the importance of milk culture and differentiation of mastitis pathogens, in order to be able to make specific recommendations in udder health control programs.

6.7 References

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CHAPTER 7

SOMATIC CELL COUNT DURING AND BETWEEN MILKINGS

by

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7.1 Abstract

The objectives of the study were to determine: 1) how sampling time between milkings affects the sensitivity and specificity of somatic cell count (SCC) as indicator for intramammary infection (IMI) status, and 2) which cells are responsible for the diurnal variation in SCC. Six Prince Edward Island, Canada, dairy herds were selected. Quarter samples for SCC were collected immediately before AM milking (PRE-AM), half-way through AM milking, immediately after AM milking, every 60 min after detachment of the milking unit, and immediately before PM milking (PRE-PM). Compared with the geometric mean SCC at PRE-AM, SCC of quarters with no IMI between milkings was higher up to 7 h after milking. PRE-PM SCC was significantly lower than the PRE-AM SCC in quarters with no IMI. Specificity of SCC at a cut-off of 200,000 or 500,000 cells/mL as indicator for IMI status declined substantially after morning milking. In quarters with elevated SCC, the proportion of polymorphonuclear leucocytes was larger immediately after milking. For accurate interpretations of SCC tests, whether by laboratory, portable SCC devices, or the California Mastitis Test, veterinarians, researchers, and udder health advisors should take milk samples immediately before milking.

7.2 Introduction

Somatic cell count is the most frequently used indicator of subclinical mastitis in dairy cattle. The most important cause of increased SCC is a bacterial infection of the mammary gland (Dohoo and Meek, 1982; Harmon, 1994). Non-bacterial factors that affect SCC include age, stage of lactation, season, stress, management, day-to-day variation, and diurnal variation. These factors are considered less important than IMI status (Dohoo and Meek, 1982; Reneau, 1986; Harmon, 1994). However, diurnal variation of SCC could have consequences for interpretation of SCC data if milk samples are collected at any time other than immediately before or during milking (Dohoo and Meek, 1982). Milk samples for SCC analysis as part of Dairy Herd Improvement programs are routinely collected at milking time. For researchers and veterinarians, sample collection during milking may not always be feasible. Furthermore, with increased use of portable somatic cell counters, milk samples are more likely to be taken in between milkings by dairy producers or their advisors. Sensitivity and specificity of SCC at a threshold of 200,000 cells/mL as indicator of presence of IMI are estimated at 73% and 86%, respectively (Dohoo and Leslie, 1991). If SCC changes after milking, a correction factor may be needed to obtain the same sensitivity and specificity for SCC as indicator of IMI.

Diurnal variation has been suggested to be the result of proportional dilution relative to milking interval, and is thought to be larger in high producing cows than in low producing cows (Reneau, 1986). The most recent study on diurnal variation that included between milking variation dates from 1967 (White and Rattray, 1965; Cullen, 1967; Smith and Schultze, 1967) and milk production has more than doubled since then.

With decreasing mean individual cow SCC and increased milk production per cow, it may be possible that the SCC decreases faster post-milking nowadays and samples that are indicative of IMI status can be taken sooner after milking.

Each somatic cell type has its own specified function in the immune response of the mammary gland: a high SCC can be the result of an increase in PMNL (Leitner et al., 2000). No studies have been reported about the fluctuation of these cells during the day synchronic with the diurnal variation of the SCC.

The objectives of the study were to determine: 1) how sampling time affects the sensitivity and specificity of SCC as an indicator of IMI status, and 2) which cells are responsible for the diurnal variation in SCC.

7.3 Materials and methods

7.3.1 *Herd and Cow Selection*

Six Prince Edward Island, Canada, dairy farms were selected that housed their lactating cows in tie-stalls and milked twice daily. Each herd was milked AM and PM with a 9 to 10 h interval, as measured from the end of AM milking to the start of PM milking. Within each herd, 9 to 11 cows were selected that had 4 milk producing udder quarters, no clinical mastitis, and a production of more than 10 kg/day. In addition, an effort was made to obtain a similar distribution in the following categories: last DHI test $\leq$ 200,000 cells/mL or > 200,000 cells/mL, first, second or third and later lactation, early ($\leq$ 100 DIM), mid (101-200 DIM) or late (> 200 DIM) lactation, and < 20, 20-30 or > 30 kg/day milk production (Table 1).

Table 1. Overview of 60 cows, which were selected on previous Dairy Herd Improvement somatic cell count (SCC), stage of lactation (DIM), parity, and daily milk production (as recorded the previous day).

	Parity			Daily milk production (kg)			
Stage of lactation	1	2	>2	10-20	20-30	30	Total
Low SCC group[1]							
10-100 DIM	1	3	1	0	2	3	5
101-200 DIM	4	2	5	1	6	4	11
201-300 DIM	4	2	3	4	5	0	9
>300 DIM	1	3	1	2	3	0	5
High SCC group[2]							
10-100 DIM	0	2	4	0	1	5	6
101-200 DIM	1	1	2	2	1	1	4
201-300 DIM	3	3	8	12	1	1	14
>300 DIM	1	3	2	3	3	0	6
Total	15	19	26	24	22	14	60

[1] SCC < 200,000 cells/mL.
[2] SCC ≥ 200,000 cells/mL.

7.3.2 *Milk Samples*

Immediately before AM milking (PRE-AM) and immediately before PM milking (PRE-PM), quarter milk samples were collected in a 60 mL plastic vial after wiping off the udder with a dry and clean paper towel and removing 3 squirts of milk. The milk sample just before AM milking was taken in duplicate. Quarter samples half-way through AM milking, immediately after AM milking, and every 60 min after detachment of the milking unit were collected in 60 mL plastic vials after removal of 3 squirts of milk. The mid-point of the AM milking was estimated based on milk production of the cow (in kg) at the previous morning milking. Sterile quarter milk samples for bacteriological analysis were collected in duplicate at PRE-AM and PRE-PM after SCC samples were taken and the teat was disinfected with a squeezed alcohol drenched cotton.

Samples for differential cell counting were collected from 20 cows on two farms (10 on each farm). At each sampling moment, 5 mL of milk was collected and poured into a sterile glass vial immediately after collection. Samples were subsequently stored in a cooler box on ice packs and transported to the laboratory, where they were stored overnight at 4°C.

7.3.3 *Laboratory Analyses*

Somatic cell count was determined within 24 h after collection of milk samples using an electronic cell counter (Fossomatic Series 400, Foss Electric A/S, Hillerød, Denmark). In total, 21 (0.7%) observations of 13 quarters in 6 cows were excluded from SCC analysis because there was an insufficient amount of milk in the sample.

Preparation of differential cell count samples and microscopic differential cell count was performed based on the techniques as described by Schröder and Hamann (2005). In detail, the milk in glass tubes was centrifuged 2 times for 10 min at 1,516 g. A fat layer was removed after the first centrifugation and after the second centrifugation the remaining fat and supernatant was removed until 0.25 mL of fluid was left. The remaining cell pellet was resuspended in the remaining 0.25 mL. From this suspension 25 μL was spread over a microscope slide. The slide was dried on a slide warmer, fixated with methanol and stained with Wright's stain using an automated stainer (HEMA-TEK ® 2000, Model 4488B, Bayer Diagnostics, Tarrytown, NY, USA). On each slide, up to 100 cells were identified, at a magnification of 1,000x.

7.3.4 *Bacteriological Analysis*

Bacteriological culture was performed according to NMC standards (Hogan et al., 1999). For each sample, the number of colony-forming units (CFU) of each bacterial species was counted. Of the pathogens that were cultured, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, other *Streptococcus* spp., and *Escherichia coli* were considered major pathogens, whereas coagulase-negative staphylococci, *Corynebacterium bovis,* enterococci, and *Bacillus* spp. were considered minor pathogens (Barkema et al., 1999). A quarter was considered infected with a major pathogen if the same organism was cultured from both PRE-AM samples. A quarter was considered to be infected with a minor pathogen if the same pathogen was cultured from both PRE-AM samples, and at least one milk sample produced ≥ 1,000 CFU/mL. If no diagnosis could be made based on the PRE-AM cultures, the PRE-PM samples were cultured and the same rules as for the PRE-AM

cultures were applied. If three or more bacterial species were cultured from a sample, the sample was considered contaminated.

Quarters were divided into three categories based on infection status: no IMI, IMI with minor pathogens, and IMI with major pathogens. If a quarter was infected with both a minor and a major pathogen, it was considered to be infected with a major pathogen.

7.3.5 *Statistical Analysis*

Data were checked for unlikely values. Separate analyses were carried out for SCC during milking (PRE-AM milking, half-way through AM milking, immediately after AM milking, and PRE-PM milking) and between milking (hours 1-9 after PRE-AM milking), as well as for the proportions of PMNL and of macrophages and monocytes during and between milking.

The SCC analyses used linear mixed models with herd fixed effects, cow random effects and a direct product correlation structure on quarters and time to account for correlations between and within quarters over time (Galecki, 1994). To approximate the normal distribution, a natural logarithmic transformation of SCC values (1,000 cells/mL) was used (Ali and Shook, 1980). The main advantage of a direct product correlation structure compared to a standard hierarchical model with a repeated measures correlation structure within quarters (Dohoo et al., 2003) is that it extends the within-quarter correlation structure to between quarter correlations. Specifically, the between milking analysis used a first-order autoregressive correlation structure for the time component, whereby correlations both within and between quarters decayed over time. A variable for parity was removed from the model because it was not significant ($P >$

0.05). Milk production was dichotomized into low and high milk producing cows at 22 kg/day, the median of the cows involved in this study. Lactation stage was divided into three categories: early (DIM $\leq$ 100), mid (100 < DIM $\leq$ 200), and late (DIM > 200). The resulting model for the difference between the natural logarithm of PRE-AM SCC and the natural logarithm of SCC at a time in the between-milking interval, the l^{th} measurement in quarter k in cow j in herd i was as follows:

$$\begin{aligned} \text{lndiffSCC}_{ijkl} = {} & \beta_0 + \beta_1 \text{hour}_l + \beta_2 \text{minor}_{ijk} + \beta_3 \text{major}_{ijk} + \beta_4 \text{himilk}_{ij} + \beta_5 \text{dim}_{ij} \\ & + \beta_6 \text{lf}_{ijk} + \beta_7 \text{lr}_{ijk} + \beta_8 \text{rf}_{ijk} \\ & + \beta_9 (\text{hour}_l * \text{minor}_{ijk}) + \beta_{10} (\text{hour}_l * \text{major}_{ijk}) \\ & + \beta_{11} (\text{hour}_l * \text{himilk}_{ij}) + \gamma_i + u_{ij} + \varepsilon_{ijkl} \end{aligned} \quad (1)$$

where β_0 is the intercept; β_1 is the regression coefficient for time after milking in hours; β_2 and β_3 are regression coefficients for infection with a minor (β_2) or major pathogen (β_3); β_4 and β_5 the regression coefficients for high milk production (β_4) and DIM (β_5); β_6 to β_8 the regression coefficients for quarters; β_9 to β_{11} the regression coefficients of the interactions between hours after milking and IMI status (β_9 and β_{10}) and between hours after milking and production level (β_{11}); γ_i is the regression coefficient for herd i; u_{ij} is the cow random effect, and ε_{ijkl} is the error term with a direct product autoregressive correlation structure on quarters and time.

For during milking SCC, a similar linear mixed model with a direct product correlation structure was used, except that the within-quarter correlations were modeled as unstructured instead of autoregressive, due to the irregular spacing in time. Pairwise comparisons of sample times within IMI levels as well as of IMI levels within sample

times were adjusted for multiple comparisons by the Bonferroni method. Non-significant effects of quarter, herd, lactation stage and high milk production ($P > 0.05$) were omitted. The model for the natural logarithm of SCC at certain moments during milking, the l^{th} measurement in quarter k in cow j in herd i was as follows:

$$\begin{aligned} \ln SCC_{ijkl} = \quad & \beta_0 + \beta_1 \, minor_{ijk} + \beta_2 \, major_{ijk} + \beta_3 \, halfway_l + \beta_4 \, postam_l + \beta_5 \, prepm_l \\ & + \beta_6 \, (minor_{ijk} * halfway_l) + \beta_7 \, (minor_{ijk} * postam_l) \\ & + \beta_8 \, (minor_{ijk} * prepm_l) + \beta_9 \, (major_{ijk} * halfway_l) \\ & + \beta_{10} \, (major_{ijk} * postam_l) + \beta_{11} \, (major_{ijk} * prepm_l) \\ & + u_{ij} + \varepsilon_{ijkl} \end{aligned} \qquad (2)$$

where β_0 is the intercept; β_1 and β_2 are regression coefficients for IMI status; β_3 to β_5 are the regression coefficients of sample moment; β_6 to β_{11} are the regression coefficients of the interaction of IMI status and sample moment, u_{ij} is the cow random effect, and ε_{ijkl} is the error term with a direct product unstructured correlation structure on quarters and time.

Using IMI status as the gold standard, sensitivity and specificity were calculated for each sampling moment for two cut-off values of SCC, i.e. 200,000 and 500,000 cells/mL.

Due to low observed proportions of lymphocytes, squamous cells, and degenerated cells, only the proportions of PMNL as well as of macrophages and monocytes (combined) were subjected to statistical analysis. All analyses were based on logistic regression models with random effects for cows and quarters as well as a first order autoregressive repeated measures correlation structure within quarters and an

extra-binomial dispersion parameter. Non-significant effects of quarter and herd ($P > 0.05$) were omitted. The models for the proportions of PMNL or of macrophages and monocytes between milkings were as follows:

$$\text{logit}(p_{ijkl}) = \beta_0 + \beta_1 \text{hiscc}_{ij} + \beta_2 \text{hour}_l + \beta_3 (\text{hour}_l * \text{hiscc}_{ij}) + u_{ij} + v_{ijk} \qquad (3)$$

and the models for the proportions of PMNL or of macrophages and monocytes among the cells identified at certain moments during milking were as follows:

$$\begin{aligned} \text{logit}(p_{ijkl}) = {} & \beta_0 + \beta_1 \text{hiscc}_{ij} + \beta_2 \text{halfway}_l + \beta_3 \text{postam}_l + \beta_4 \text{prepm}_l \\ & + \beta_5 (\text{halfway}_l * \text{hiscc}_{ij}) + \beta_6 (\text{postam}_l * \text{hiscc}_{ij}) \\ & + \beta_7 (\text{prepm}_l * \text{hiscc}_{ij}) + u_{ij} + v_{ijk} \end{aligned} \qquad (4)$$

where $\text{logit}(p) = \ln(p / (1-p))$; p_{ijkl} is the proportion of a cell type in a sample taken at sampling moment l from quarter k within cow j within herd i; β_0 is the intercept; β_1 is the regression coefficient for quarters with high SCC (> 200,000 cells/mL); β_2 and β_3 in model (3) are the regression coefficients for time after milking in hours (β_2) and its interaction with high SCC; β_2 to β_7 in model (4) are the regression coefficients for sample moments (β_2 to β_4) and their interaction with high SCC; u_{ij} is the cow random effect, and v_{ijk} the quarter random effect.

All mixed model analyses were carried out using SAS software (SAS for Windows, version 9.1; SAS Institute, Inc., Cary, NC): the linear mixed models by the MIXED procedure, and the generalized linear mixed by the experimental GLIMMIX procedure. All other statistical calculations were carried out using Stata software

(Intercooled Stata for Windows, version 8.2; Stata Corporation, College Station, Texas, USA).

7.4 Results

7.4.1 *Culture results*

In total, 17 (7.1%) quarters were infected with major pathogens: 11 *Staph. aureus,* 1 mixed infection with *Staph. aureus* and *Strep. dysgalactiae*, 1 *Strep. uberis*, 3 *Streptococcus* spp. other than *Strep. agalactiae*, *Strep. uberis,* or *Strep. dysgalactiae*, and 1 *E. coli*. Thirty-one (12%) quarters were infected with minor pathogens: 12 coagulase-negative staphylococci and 19 *C. bovis*. Two samples were considered contaminated.

7.4.2 *Somatic Cell Counts*

The geometric mean SCC of all quarters (n = 240) included in the study was 101,000 cells/mL (ranging from 5,000 to 7,677,000 cells/mL) at PRE-AM and increased sharply after the AM milking (Fig. 1) to a maximum of 322,000 cells/mL 1 h after milking (ranging from 15,000 to 8,136,000 cells/mL). Compared with the geometric mean SCC at PRE-AM, SCC of post-milking samples was higher until 7 h after milking (Fig. 1). For example, substituting the estimated coefficients of Table 2 in model (1), the natural logarithm of the right front quarter with no IMI in a high producing, mid-lactation cow in herd 2 would be elevated at 3 h after milking (compared to PRE-AM milking) by an amount of 1.179 + (-0.144 * 3) + 0 + (0.604 * 1) + 0.479 + (-0.162) + 0 + (3 * -0.052) + (-0.205) = 1.47 (Table 2).

Table 2. Mixed model of the difference between natural logarithm of the pre-AM milking somatic cell count (SCC) and the natural logarithm of the between-milking SCC of 240 quarters of 60 cows.

	Coefficient β	SE[1]	*P*-value
Intercept	1.179	0.174	< 0.01
Quarter			< 0.01
Left Front	Ref.[2]	-	
Right Front	-0.162	0.074	
Left Rear	-0.243	0.049	
Right Rear	-0.315	0.066	
Hour[3]	-0.144	0.013	< 0.01
Intramammary infection			< 0.01
None	Ref.	-	
Minor pathogen	-0.077	0.123	
Major pathogen	0.764	0.175	
Milk[4] > 22 kg/day	0.604	0.168	< 0.01
Stage of lactation			< 0.01
Early (< 101 DIM[5])	Ref.	-	
Mid (101-200 DIM)	0.479	0.135	
Late (> 200 DIM)	0.407	0.141	
Hour x Intramammary infection			< 0.01
None	Ref.	-	
Minor pathogen	0.012	0.019	
Major pathogen	0.083	0.026	
Hour x Milk > 22 kg/day	-0.052	0.018	< 0.01
Herd			0.05
Herd 1	Ref.	-	
Herd 2	-0.205	0.162	
Herd 3	-0.131	0.177	
Herd 4	-0.260	0.143	
Herd 5	-0.482	0.160	
Herd 6	-0.284	0.149	

[1]SE = Standard error.
[2]Ref. = Reference category.
[3]Hour after end of milking as continuous variable.
[4]Milk production per day dichotomized at median.
[5]DIM = Days in milk.

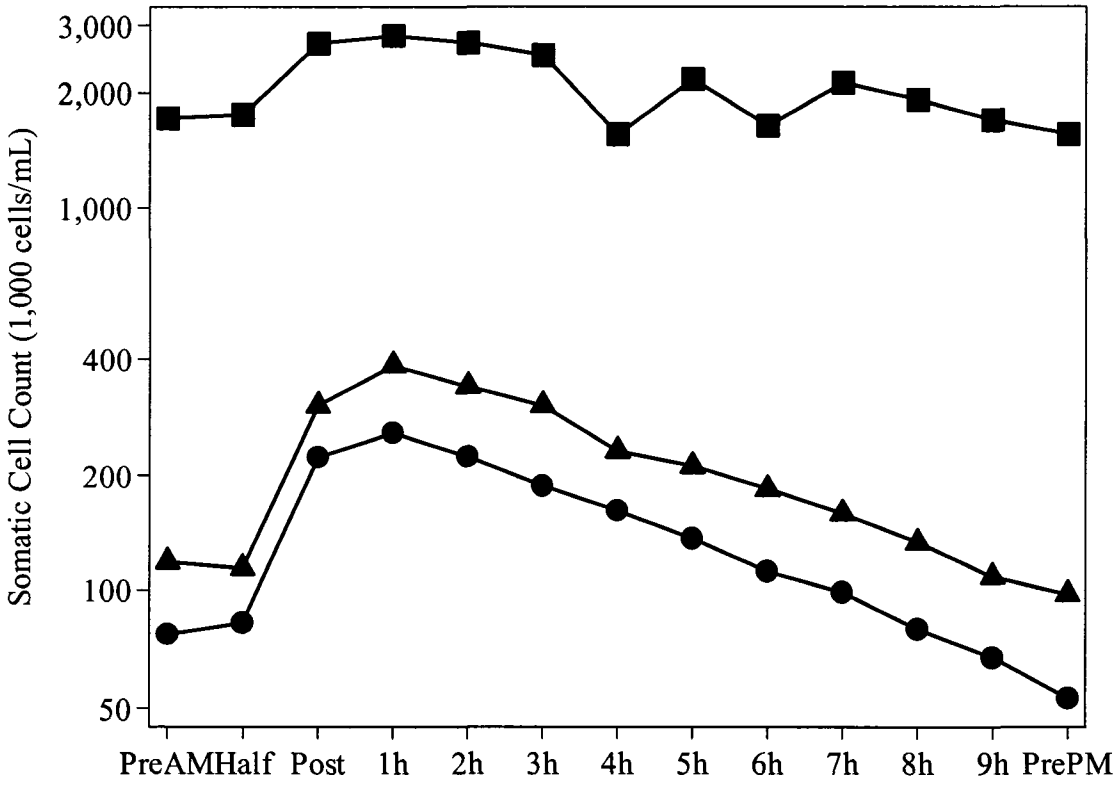

Figure 1. Geometric mean quarter (n=240) somatic cell count during and between milkings for quarters without an intramammary infection (●; n=192), quarters with an infection with minor pathogens (▲; n=31), and quarters with an infection with major infections (■; n=17).

The mean SCC of quarters with a major pathogen IMI was higher than for quarters with minor pathogen IMI or no IMI and did not increase much after the AM milking (Fig.1; page 202; Table 2; page 203). Somatic cell counts of quarters with a minor pathogen IMI differed only little from quarters with no IMI (Fig. 1; page 202; Table 2; page 203). Compared with low producing cows the difference between PRE-AM SCC and SCC in high producing cows 1 h after the AM milking was bigger and declined faster after that time (Table 2; page 203). The difference between SCC PRE-AM and SCC between milkings was smaller in quarters of cows in early lactation than in cows later in lactation (Table 2; page 203). Compared with rear quarters, front quarters had larger differences in SCC between PRE-AM and SCC between milkings; in addition, left front quarters had larger differences than right front quarters and left rear quarters had larger differences than right rear quarters (Table 2; page 203).

For quarters with no IMI, the PRE-AM SCC increased from a least squares estimate of 75,000 cells/mL to an estimated post milking SCC level of 220,000 cells/mL ($P < 0.01$, Table 3). The SCC levels at half-way milking were not significantly different from the PRE-AM level (Table 3). At PRE-PM, SCC was with 53,000 cells/mL for quarters with no IMI lower than PRE-AM SCC ($P < 0.01$) (Table 3). For quarters with a major pathogen IMI, PRE-PM (1,509,000 cells/mL) and half-way milking SCC (1,634,000 cells/mL) were not significantly different from PRE-AM SCC (1,390,000 cells/mL), whereas post milking SCC (2,877,000 cells/mL) was different.

The sensitivity of SCC as an indicator of major pathogen IMI at a cut-off of 200,000 cells/mL was 100% at almost any moment of sampling (Fig. 2). The specificity of SCC as an indicator of major pathogen IMI dropped from 73% (95% exact binomial confidence interval (CI): 67-79%) pre-milking to 34% (95% CI: 28-41%) 1 h after

Table 3. Mixed model of the natural logarithm of PRE-AM milking somatic cell count (SCC), and the natural logarithm of the between-milking SCC of 240 quarters of 60 cows.

	Coefficient β	SE[1]	*P*-value
Intercept	4.322	0.144	
Intramammary infection (IMI)			< 0.01
None	Ref.[2]	-	
Minor pathogen	0.660	0.232	
Major pathogen	2.915	0.304	
Sampling moment			< 0.01
PRE-AM[3]	Ref.	-	
Half-way	0.107	0.061	
POST-AM	1.071	0.072	
PRE-PM[4]	-0.349	0.080	
IMI x sampling moment			< 0.01
Minor pathogen x PRE-AM	Ref.	-	
Minor pathogen x Half-way	-0.130	0.109	
Minor pathogen x POST-AM	-0.104	0.130	
Minor pathogen x PRE-PM	0.066	0.144	
Major pathogen x PRE-AM	Ref.	-	
Major pathogen x Half-way	0.054	0.142	
Major pathogen x POST-AM	-0.344	0.170	
Major pathogen x PRE-PM	0.431	0.188	

[1]SE = Standard error.
[2]Ref. = Reference category.
[3]PRE-AM = Sampling moment is immediately before AM milking.
[4]PRE-PM = Sampling moment is immediately before PM milking.

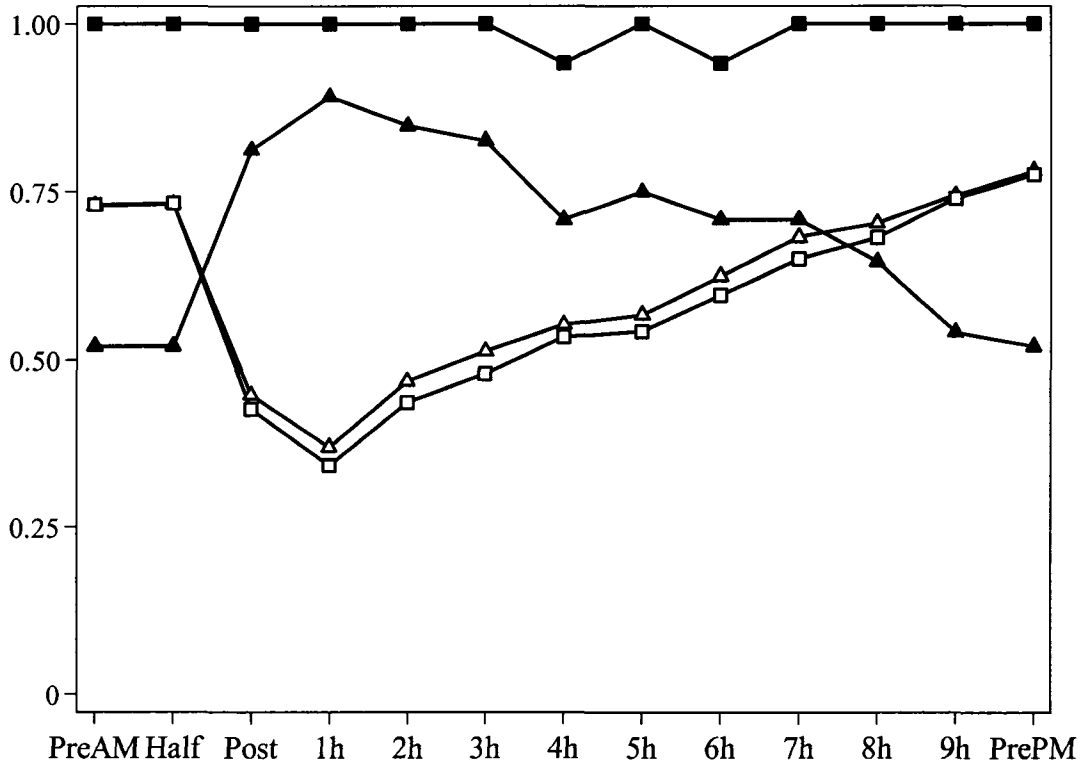

Figure 2. Sensitivity (■) and specificity (□) of somatic cell count at the threshold of 200,000 cells/mL to determine an intramammary infection with major pathogens or for any intramammary infection (sensitivity = ▲; specificity = Δ) during and between milkings.

milking and then increased slowly until the PM milking (Fig. 2; page 206). When both major and minor pathogen IMI were considered, the specificity of SCC followed a similar pattern but the sensitivity was much lower. The specificity was 37% (95% CI: 30-44%) 1 h after milking, while the sensitivity started at 52% (95% CI: 37-67%) PRE-AM, increased to 89% (95% CI: 76-96%) 1 h after milking and slowly declined back to 52% (95% CI: 37-67%) at the PM milking (Fig. 2; page 206).

The sensitivity to determine an IMI with a major pathogen using a cut-off value of 500,000 cells/mL was 82% and higher at any moment of the sampling period (Fig. 3). The specificity at a cut-off value of 500,000 cells/mL at PRE-AM was initially 91% (95% CI: 86–94%), and dropped to 70% (95% CI: 63-76%) at 1 h after milking (Fig. 3). The specificity of SCC to determine any IMI at the cut-off value of 500,000 cells/mL followed a similar pattern as that of major pathogens, while the sensitivity was 40% (95% CI: 26-55%) at PRE-AM, reached its highest value at 65% (95% CI: 50-79%) at 1 h after milking and decreased slowly to pre-milking levels up to the PM milking (Fig. 3).

7.4.3 *Cell Differentiation*

The mean number of cells counted per slide was 81. In 70.1% of slides (n = 1,036) more than 90 cells were counted, and in 3.7% less than 10. The proportions of macrophages and monocytes, PMNL, lymphocytes, squamous cells, and degenerated cells in milk samples with low SCC (≤ 200,000 cells/mL) and taken after removal of foremilk were 66, 22, 0.3, 7.5, and 4.2%, respectively, compared with 54, 38, 1.1, 3.4, and 2.6% in milk samples with elevated SCC (> 200,000 cells/mL) (Fig. 4). In low SCC quarters, the proportions of PMNL did not change, while in high SCC quarters the proportion of PMNL was larger than in low SCC quarters at any time, but decreased

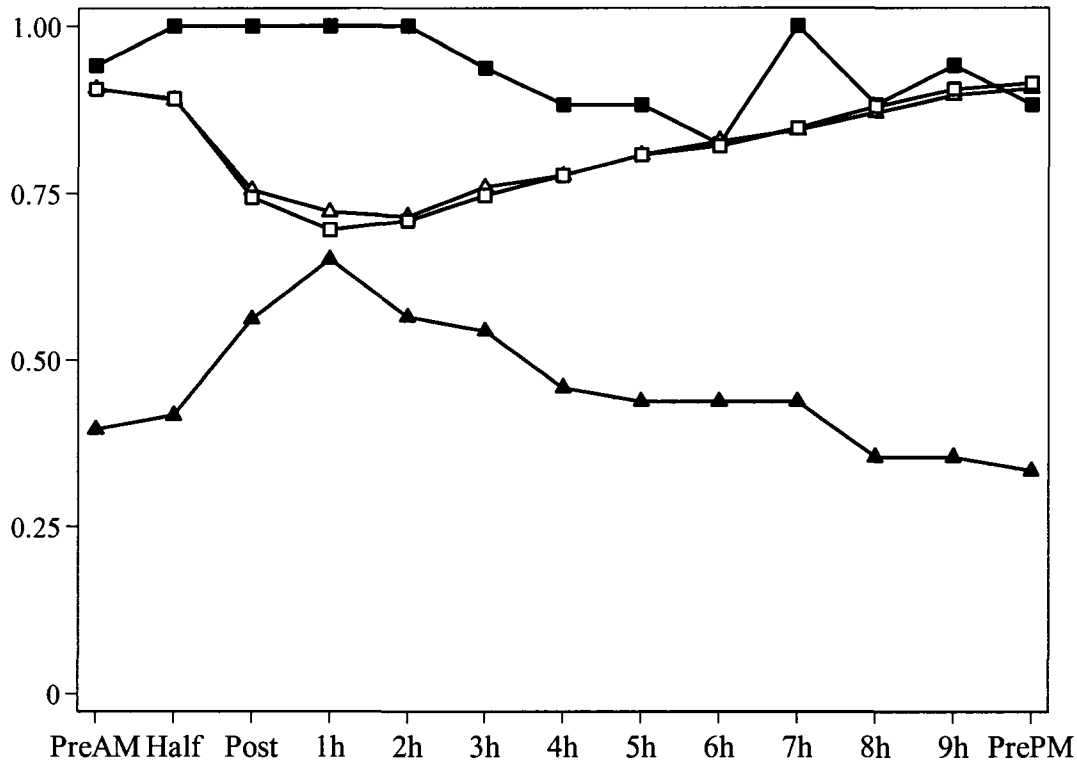

Figure 3. Sensitivity (■) and specificity (□) of somatic cell count at the threshold of 500,000 cells/mL to determine an intramammary infection with major pathogens or for any intramammary infection (sensitivity = ▲; specificity = Δ) during and between milkings.

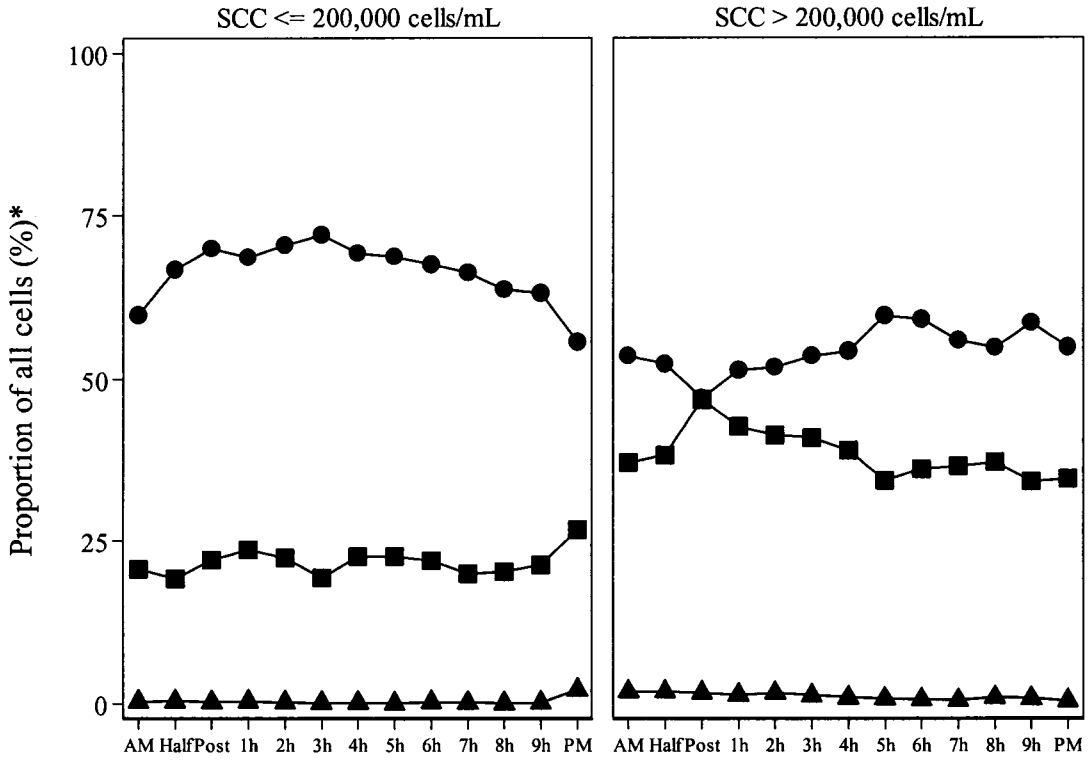

Figure 4. Proportions of macrophages (●), polymorphonuclear leukocytes (■), and lymphocytes (▲) during and between milkings for quarters with SCC ≤ 200,000 cells/mL (n = 56) SCC > 200,000 cells/mL (n = 24). * Proportions of squamous cells and degenerated cells were omitted in the figure.

towards the PM milking (Fig. 4; page 209; Table 4). The proportion of macrophages and monocytes decreased slightly between milkings in low SCC quarters and were larger at any time than in high SCC quarters; in high SCC quarters, these proportions increased over time between milkings (Fig. 4; page 209; Table 4). The proportions of PMNL were largest in high SCC quarters (except at PRE-PM), but were only significantly elevated relative to PRE-AM immediately after AM milking (Fig. 4; page 209; Table 4). In low SCC quarters, the proportions of macrophages and monocytes were significantly larger in the half-way and post-AM milking samples compared with PRE-AM, and they were at most sampling moments smaller in high SCC quarters (Fig 4; page 209; Table 4).

7.5 Discussion

Somatic cell counts in quarter milk samples changed considerably during the day. The observed diurnal variation of SCC was in agreement with earlier research (White and Rattray, 1965; White and Rattray, 1967; Cullen, 1967; Smith and Schultze, 1967). There are several possible scenarios in which the diurnal variation of SCC could be explained: 1) decreasing cell influx and constant milk influx; 2) constant cell influx and increasing milk influx; and 3) combination of decreasing cell influx and increasing milk influx in between milkings. Several studies have demonstrated that milk flow in the udder cistern increases from 4 h after milking onwards (Knight et al., 1994; Bruckmaier, 2005). Other studies have shown that the proportion of PMNL in the blood supply to the udder changes in the time after milking (Paape and Guidry, 1969; Knight et al., 1994). Although not proven, but based on the reported increased proportion of

Variable	PMNL			Macrophages and monocytes		
BETWEEN MILKING						
	β	SE	*P*	β	SE	*P*
Intercept	-1.288	0.217		0.898	0.167	
High SCC[2]	0.756	0.204	< 0.01	-0.772	0.158	< 0.01
Hour[3]	-0.004	0.013	< 0.01	-0.026	0.012	0.21
High SCC x hour	-0.051	0.020	0.01	0.077	0.019	< 0.01
DURING MILKING						
	β	SE	*P*	β	SE	*P*
Intercept	-1.446	0.307		0.440	0.167	
High SCC	0.548	0.140	< 0.01	-0.071	0.154	< 0.01
Sample moment			< 0.01			0.07
PRE-AM[4]	Ref.	-		Ref.	-	
Half-way[5]	-0.061	0.099		0.391	0.115	
POST-AM[6]	0.228	0.099		0.391	0.111	
PRE-PM[7]	0.296	0.114		-0.123	0.125	
High SCC x Sample moment			< 0.01			< 0.01
PRE-AM	Ref.	-		Ref.	-	
Half-way	0.251	0.191		-0.425	0.186	
POST-AM	0.290	0.194		-0.708	0.183	
PRE-PM	-0.488	0.224		0.076	0.208	

[1]SE = Standard error.
[2]High Somatic Cell Count (> 200,000 cells/mL).
[3]Time after detachment of unit at AM milking.
[4]PRE-AM = Sample taken immediately before AM milking.
[5]Half-way = Sample taken half-way AM milking.
[6]POST-AM = Sample taken immediately after detachment of unit at AM Milking
[7]PRE-PM = Sample taken immediately before PM milking.

PMNL in the udder's blood supply (Paape and Guidry, 1969), we hypothesize that changes in SCC in between milkings are possibly caused by relatively high influx of cells shortly after milking, and a subsequent dilution effect due to the increased milk influx hours later.

Handling of the cow and quarters 3 times during milking and every hour thereafter until the PM milking could have affected SCC somewhat as mechanical stimulation of the udder seems to be associated with increased SCC (Rasmussen et al., 2005). Because the proportion of squamous cells in milk was small and constant in our study, we believed that mechanical stimulation only had a minimal effect and would not have influenced the outcome of our study. Milk leakage between sampling was occasionally seen, but the authors did not consider this as a major influence on SCC, and nor on the outcomes of the study.

Significant differences in SCC, associated with quarter position, were observed between quarters within a cow similar to other studies. Incidence of clinical mastitis is higher in rear quarters than in front quarters (Batra et al., 1977; Adkinson et al., 1993; Barkema et al., 1997). Right quarters are associated with higher incidence of clinical mastitis (Barkema et al., 1997) and subclinical mastitis (Zadoks et al., 2001) than left quarters. A recent study by Berry and Meaney (2006) found that subclinical mastitis, defined as SCC > 250,000 cells/mL, occurred more often than expected in rear quarters than in front quarters.

The diurnal variation in SCC has consequences for the use of SCC as indicator of IMI status. The sensitivity and specificity of SCC was explored for two thresholds: 200,000 cells/ml and 500,000 cells/ml. Based on sensitivity and specificity, a cut-off value of 200,000 cells/mL is considered the most appropriate threshold for diagnosis of

IMI with major pathogens (Dohoo and Leslie, 1991; Schukken et al., 2003). A cut-off level of 500,000 cells/mL was also considered because it reflects the recommended threshold for diagnosis of IMI with the California Mastitis Test (CMT) (Casura et al., 1995). For both thresholds, the sensitivity of finding an IMI with major pathogens remains high. Shortly after the AM milking, SCC is relatively high, resulting in a high proportion of false positive diagnoses of IMI, and low test-specificity. Our results suggest that a correction formula may be developed for SCC values between milkings based on a broader study population than the present.

No difference was found between SCC PRE-AM and halfway through the AM milking. A higher SCC in strict foremilk, defined as the first 2 stripped jets of milk, than cisternal or alveolar milk fractions taken from quarters with SCC > 100,000 cells/mL has been reported (Sarikaya and Bruckmaier, 2006). We collected samples only after the foremilk, in our case the first 3 strippings of milk, were removed. By contrast, post-milking SCC was much higher than PRE-AM SCC. This difference may be the result of the start of influx of white blood cells before the end of milking. Another explanation is that the cow was milked out completely and the sample, which was taken after the removal of the unit, contained the first milk produced after milking with a lot of cells. SCC was considerably lower at PRE-PM milking than at PRE-AM milking. Earlier studies reported higher mean SCC PRE-PM than PRE-AM (White and Rattray, 1965; White and Rattray, 1967; Cullen, 1967; Smith and Schultze, 1967; Reneau, 1986; Nielsen et al, 2005). This discrepancy could be the result of the herds in the present study having 9 to 10 h between the AM and PM milking, whereas some studies had only 7 h between the start of AM and PM milking (Cullen, 1967; Smith and Schultze, 1967; Reneau, 1986). Nielsen et al. (2005) reported higher SCC during most of the milking

process at 6 h milking intervals compared with 12 h milking intervals. At 6 to 7 h after the AM milking in our study, SCC was still higher than PRE-AM. By contrast, Weiss et al. (2002) used proportional sampling and did not find any difference when cows were milked at various intervals.

The proportions of macrophages, PMNL, lymphocytes, squamous cells, and degenerated cells were similar to those reported earlier for quarters of normal healthy cows (Kurzhals et al., 1985). The proportion of PMNL in quarters with an elevated SCC was almost twice the proportion of PMNL in milk from quarters with a low SCC. The larger proportion of PMNL can be explained by the chemotactical mobilisation of PMNL induced by macrophages in the udder in response to an IMI. In quarters with elevated SCC, relative proportion of PMNL was larger shortly after milking than later on (Fig 4).

The finding that PRE-PM SCC was significantly lower than PRE-AM SCC, has implications for the interpretation of DHI data and sampling. In some countries, for example in Canada, DHI organizations sometimes alternate herd sampling between AM and PM milking. This means that between measures, cows have on average higher or lower SCC, depending on the time of sampling. Our results also imply that samples collected from all cows in herds that are not enrolled in a DHI program on moments other than pre-milking do not reflect the average herd SCC and the average would not be a good predictor for the bulk milk SCC.

7.6 Conclusion

In quarter samples collected between milkings, SCC is not a reliable indicator of the IMI status. Differential cell ratios did not change much during the day in quarters with low SCC, and therefore no specific cell type is attributing to the SCC fluctuation between milkings in these quarters. Quarters with an elevated SCC however, showed a relatively higher proportion of PMNL shortly after milking, followed by gradual decline to pre-milking levels. The proportion of macrophages mirrored this pattern. To be able to make optimal interpretations of SCC tests, whether by laboratory, portable SCC devices, or CMT, veterinarians, researchers, and udder health advisors should take the milk samples immediately before milking.

7.7 Acknowledgements

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CHAPTER 8

GENERAL DISCUSSION

8.1 Introduction

The reason for initiation of the studies described in this thesis is that the Canadian Bovine Mastitis Research Network (CBMRN; www.mastitisnetwork.org) needed to acquire knowledge of the distribution of mastitis pathogens across Canada to give direction to its research proposals before starting projects to improve the udder health status of the national dairy herd. The aim of the studies described in this thesis was therefore to gain insight into the current mastitis situation on Canadian dairy farms. The incidence rate of clinical mastitis (IRCM) was studied in relation to the different barn types in which lactating cows are housed and in relation to geographical regions of Canada in Chapter 2. In Chapter 3, the association of risk factors with the overall and pathogen-specific IRCM were reported. In order to have an estimate of the adoption of management practices on Canadian dairy farms and the herd-level prevalence of contagious mastitis pathogens, the study described in Chapter 4 was conducted. Because frozen milk samples were used in this study, and a very low prevalence of *Mycoplasma* and *Streptococcus agalactiae* was found, a reduced probability of isolating *Mycoplasma* and *Strep. agalactiae* was suspected. Therefore, another study was carried out using fresh bulk milk samples from Prince Edward Island (PEI) dairy herds (Chapter 5). Geographical difference and differences in adoption of management practices caused variation in IRCM, but seasonal variation could impact IRCM and other udder health parameters too. In Chapter 6, the magnitude of the impact of season on bulk milk somatic cell count (SCC), new and chronic SCCs and the pathogen-specific IRCM was described. Finally, if control programs were to be implemented, part of that would be the identification of intramammary infections (IMI) through detecting cows with

elevated SCC (> 200,000 cells/mL). However, a significant diurnal variation in SCC was found. When using a cut-off of a SCC of 200,000 cells/mL 1 to 4 h after milking a significantly higher proportion of high SCC will be false-positive for IMI than when using samples collected before milking. The change of SCC and which factors affect this change during and between milking was studied in Chapter 7.

8.2 Bulk milk

Increasing awareness of public health and food safety forces the dairy industry to produce high quality dairy products. Bulk milk SCC (BMSCC) is a key milk quality element. Worldwide, regulatory limits are in place for BMSCC. The current limit that has been set in Canada is 500,000 cells/mL, whereas the European Union, Australia and New Zealand have a limit of 400,000 cells/mL, and in the U.S.A. it is 750,000 cells/mL. Regulatory limits only came in place during the last 4 decades. They have proven to be effective in reducing BMSCC. National average BMSCC has declined considerable since the introduction of these limits. In Ontario, Canada, a 6-year stepwise system to decrease BMSCC from 800,000 to 500,000 cells/mL was introduced in 1989. There is a documented statistically significant decline in the average BMSCC during that time in Ontario (Fig. 1) (Schukken et al., 1992; Schukken et al., 1993). However, significant seasonal variation still exists (Fig. 1) (Schukken et al., 1992). The seasonal pattern of BMSCC in Canadian dairy herds is similar to that found in other countries, e.g. The Netherlands (Chapter 6) (Sargeant et al., 1998a; Norman et al., 2000; Green et al., 2006). Because a large good quality dataset was available from 300 Dutch dairy herds, this

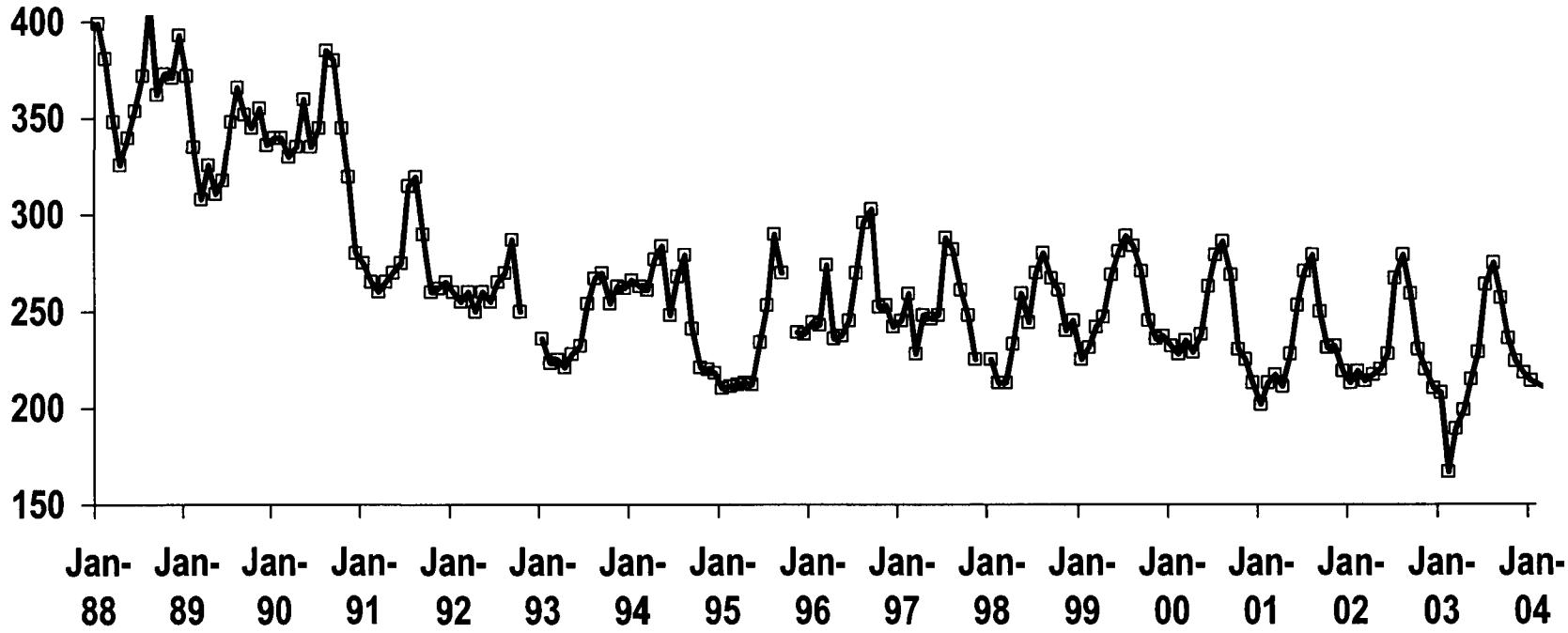

Figure 1. Bulk milk somatic cell counts for all herds in Ontario for the period January 1988 to January 2004. Figure provided by Dr. D. F. Kelton, Dept. Population Medicine, Ontario Veterinary College, Guelph, ON, Canada.

dataset was used to study the effect of season on BMSCC and other udder health parameters. This study showed that most individual mastitis pathogens had distinct seasonal IRCM pattern, suggesting that an increased incidence of subclinical IMI caused by *Strep. uberis* or *Staph. aureus* in spring and summer could be responsible for the seasonal increase in BMSCC in Canada. This peak in *Staph. aureus* and *Strep. uberis* IMI has been found in Norway as well (Østerås et al., 2006). Sargeant et al. (1998a) classified herds by BMSCC category in 1985, and in the 9 following years it appeared that herds with high BMSCC were more likely to leave the industry. A premium system for low BMSCC (< 250,000 cells/mL) has been introduced in British Columbia in 2001, while some processors in British Columbia already had a bonus system in place long before that. British Columbia has an average BMSCC just over 150,000 cells/mL, whereas the other provinces have average BMSCC greater than 200,000 cells/mL (Fig. 2). It is obvious that premium and penalty systems have an impact on BMSCC. However, the change in BMSCC does not automatically imply that the average individual cow SCC at the herd level has decreased. Farms with high BMSCC cease farming operations or more cows with elevated individual cow SCC are being kept out of the bulk tank. Additionally, not implementing generally accepted mastitis prevention practices, such as dry cow therapy, segregation of infected cows, and post-milking teat disinfection are associated with increased BMSCC (Erskine et al., 1987; Hutton et al., 1990; Barkema et al., 1998a). Therefore, reduction of BMSCC has been caused by both survival of low BMSCC herds and high BMSCC herds leaving the industry, as well as changes in management practices due to implementation of the 5-point (and later 10-point) mastitis control program. Following British Columbia, PEI has recently

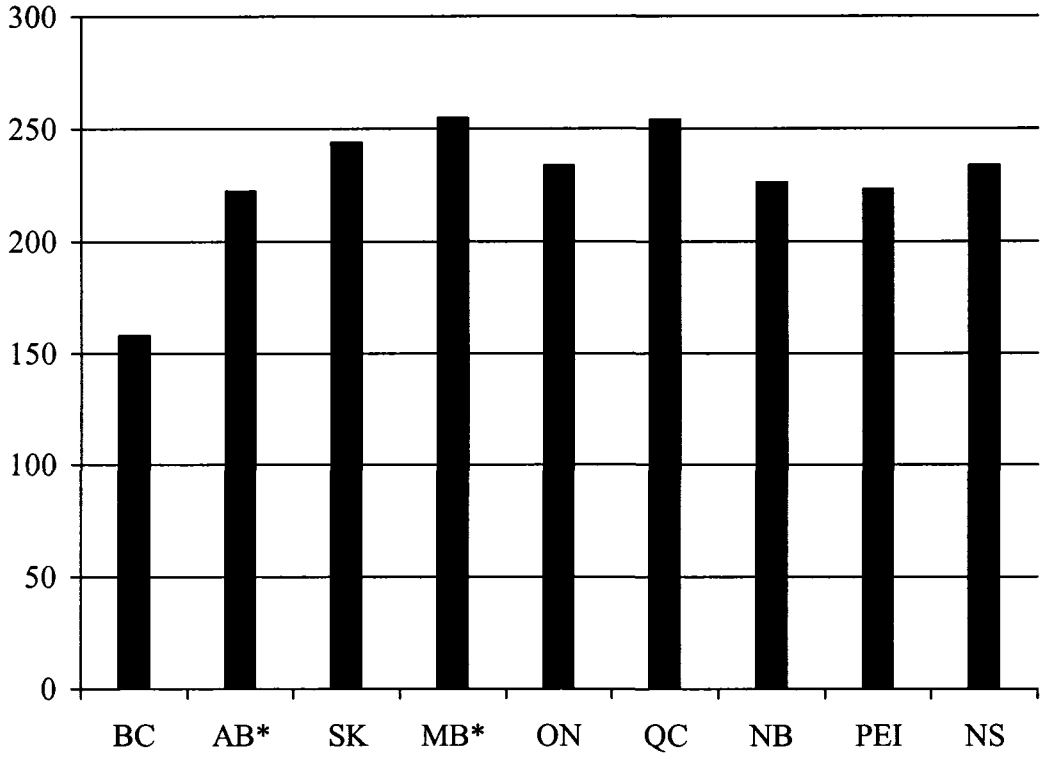

Figure 2. Average bulk milk somatic cell counts for 9 Canadian provinces in December 2006.
Source: Canadian Dairy Commission (CDC).
(http://www.dairyinfo.gc.ca/_english/dff/dff_2/dff_2c_e.htm; last visited March 17, 2007)
* Most recent available data from 2003.

implemented a similar bonus system for quality bulk milk because of the above mentioned reasons.

Mastitis pathogens that are associated with elevated BMSCC are *Strep. agalactiae*, *Staph. aureus*, *Mycoplasma* spp., *Strep. uberis*, and *Strep. dysgalactiae* (Keefe, 1997; Barkema et al., 1998b; Zadoks et al., 2001; Jayarao et al., 2004; Fox et al., 2005; Olde Riekerink et al., 2006). In Finland, BMSCC declined from 320,000 in 1990 to 180,000 cells/mL in 1995 (Myllys et al., 1998). In the same period prevalence of mastitis pathogens in subclinical mastitis has changed dramatically. *Staphylococcus aureus* prevalence declined from 31 to 17% of all isolations in that period, *Strep. agalactiae* and *Strep. dysgalactiae* prevalence declined, whereas coagulase-negative staphylococci prevalence increased (Myllys et al., 1998; Pitkala et al., 2004). Because BMSCC also decreased in Canada, the prevalence of pathogen-specific IMI will most likely have changed. This may also result in a change of pathogen-specific prevalence of contagious pathogens in bulk milk samples.

The random sample of dairy herds in the bulk milk study resulted in an estimate of the prevalence of contagious mastitis pathogens in Canada (Chapter 4 and 5). *Staphylococcus aureus* was present on 73% of the Canadian dairy farms, and based on statistical projections is likely present on all dairy farms (Chapter 5). The province with the lowest BMSCC, British Columbia, also had the lowest prevalence of *Staph. aureus* in bulk milk – but given their larger herd size, the dilution of one or two SA positive cows may have generated false negative bulk tank culture results. *Streptococcus agalactiae* was isolated in less than 1% of the bulk milk samples and most of the herds that were *Strep. agalactiae*-positive were located in Québec. As discussed before, *Strep. agalactiae* may become an eradicated pathogen in Canadian dairy cow's udders in the

near future if the current trend continues. Clearly, the contagious mastitis prevention and control program (the 5-point plan) has worked well in reducing the prevalence of *Strep. agalactiae* IMI and also *Staph. aureus* IMI (Bradley, 2002).

When performing a herd screening, occasionally an isolated *Strep. agalactiae* IMI is detected. Particularly in low BMSCC herds this pathogen can be of human origin. Because these human *Strep. agalactiae* strains are neither very virulent nor contagious (Dogan et al., 2005), no action is needed. Additionally, most of the herds that have eradicated *Strep. agalactiae* from the dairy herd, have not done this intentionally. Therefore, if an isolated case of *Strep. agalactiae* is found, assuring that the contagious mastitis control and prevention plan is followed will be the best plan to prevent exacerbation of the situation. Monitoring of the subclinical udder health situation will, however, be necessary. This can be done cost-effectively using repeated bulk milk samples.

The correlation of BMSCC and prevalence of *Staph. aureus* IMI is high. Measures to decrease BMSCC will decrease the prevalence of this pathogen. Penalty and bonus programs will, therefore, work well to decrease the prevalence of *Staph. aureus* IMI. Although four decades old, the 5-point plan is still a good tool to control *Staph. aureus*, and most herds that have a high BMSCC and/or prevalence of *Staph. aureus* do not follow this plan completely. It is therefore questionable whether a lot of money should be spent on farms that do not wish to follow proven measures to control BMSCC.

From observations in veterinary diagnostic laboratories in Canada, it was expected that *Mycoplasma* spp. would be present in a small proportion of herds, but so far no studies have actually targeted *Mycoplasma* prevalence or incidence since the

study in Ontario performed in 1972 (Ruhnke et al., 1976). Because no *Mycoplasma* spp. were isolated in the nationwide bulk tank milk sample study, we questioned whether this was the result of the method we were using. Biddle et al. (2004) found that frozen storage and thawing of milk samples has a negative impact on the recovery of *Mycoplasma* spp. in the milk. Additionally, if a minority of cows are infected, they can shed *Mycoplasma* below the detection threshold (Fox et al., 2005). It was decided to initiate another study (Chapter 5) in which we estimated the herd-level prevalence of contagious mastitis pathogens in fresh bulk milk samples from all dairy farms on PEI. Approximately the same prevalence of *Staph. aureus* was found in the PEI herds as in the national random sample of herds, suggesting that the results on these herds may be generalized to the national dairy herd. In our study with fresh bulk milk samples from PEI we found a prevalence of 2% of herds with *Mycoplasma* spp. in their bulk milk. However, if in Canada, as is found in the US (González et al., 1992; Fox et al., 2003), prevalence of *Mycoplasma* spp. IMI increases with herd size, the prevalence may be higher in the western Canadian provinces than on PEI.

Streptococcus agalactiae prevalence was as low, especially compared with a similar study in the same geographical region, namely PEI, and in the same population of dairy herds approximately 10 years earlier (Keefe et al., 1997), in which *Strep. agalactiae* was present in 14% of the PEI dairy herds, indicating very strongly that distribution of mastitis pathogens has changed over time. A national quarter-level IMI prevalence study, as has been done in some European countries, would be needed to determine the Canadian subclinical mastitis situation.

8.3 Clinical mastitis

From the results of this thesis (Chapter 2 and 4) it is clear that mastitis is still an important disease on Canadian dairy farms. The estimated IRCM of 22 clinical mastitis cases per 100 cow-years (Chapter 2) was within the range of IRCM reported by others (Wilesmith et al., 1986; Erskine et al., 1988; Schukken et al., 1989b; Barkema et al., 1998b). The reported IRCM also falls into the range of IRCM found by other authors in Canada. Sargeant et al. (1998b) and McLaren (2006) estimates were similar, those of Van Dorp et al. (1999) were much lower and Meek et al. (1986) were higher. Based on this study we cannot determine if the national IRCM is reduced, stayed the same, or increased over the past decade. Studies in other countries were not unidirectional either: In Finland the prevalence of mastitis continued to decrease (Pitkala et al., 2004), whereas in the U.K. the IRCM seemed to be constant at the same high level for many years now (Bradley et al., 2007).

The organism that was the most important cause of clinical mastitis was *Staphylococcus aureus* followed by *E. coli*, *Strep. uberis*, and coagulase-negative staphylococci (CNS) (Chapter 2). Coliforms were most often isolated from cases of clinical mastitis in a study in Ontario (Sargeant et al., 1998b), although further differentiation was not performed. *Klebsiella* spp. were the fifth most frequently isolated pathogens. It has been stated that *Klebsiella* incidence in North America is not only higher than in Europe, it is also an emerging pathogen (Roberson et al., 2004; Zadoks and Munoz, 2007). However, particularly farms in Québec, Ontario and Atlantic Canada, farm in a different way than US farms and is probably more similar to the Western-European situation. As a result, the pathogen distribution of Western

Canadian dairy farms is similar to herds with a low BMSCC in the US (Erskine et al., 1988). In the other regions of Canada the distribution is similar to what is found in European studies (Barkema et al., 1998b).

Culture-negative milk samples represented a large part (43%) of the milk sample culture results (Chapter 2 and 3). Culture-negative results are often attributed to either *E. coli* (Smith and Hogan, 1993) or *Staph. aureus* (Sears et al., 1990). The distribution of culture-negative IRCM was strikingly similar to *E. coli* IRCM, which strongly suggests that a high proportion of the culture-negative clinical mastitis cases were caused by *E. coli*, and that this pathogen was not present or viable in the milk sample collected or did not survive the frozen storage before culture (Schukken et al., 1989a; Zorah et al., 1993).

From this study it became clear that there were 3 important factors which have an impact on overall IRCM and pathogen-specific IRCM, the geographical region, barn-type (Chapter 2), and season (Chapter 6).

Overall IRCM and pathogen-specific IRCM differed per geographical region in Canada, although the province-specific IRCM in this thesis (Chapter 2) should be interpret with caution, because the number of dairy farms per province were not very large and the herds were not randomly selected. Selection criteria could have been different per province or more precisely per coordinator, who was often a practicing veterinarian or a Canadian Quality Milk Program coordinator. Some could have selected more dairy farms that had mastitis problems, others could have selected more progressive farms which had a low IRCM. The selected herds, therefore, do not necessarily represent the national herd average IRCM.

The type of barn used to house lactating cows was also important for the prevailing mastitis pathogens and IRCM on that farm. Traditionally, smaller tie-stall herds have more contagious mastitis, whereas larger free-stall herds have to face more environmental mastitis (Shpigel et al., 1998; Pyörälä, 2002).

The third external factor that is difficulty to manage is the environmental influence of season. As we reported in Chapter 6, distribution and IRCM of several pathogens can be different at various times of the year. However, care should be taken with the interpretation of these results for the Canadian situation. This study was performed on a dataset of Dutch dairy herds, and are not necessarily applicable to the Canadian situation. In the current study there were fewer herds and a larger proportion of herds were totally or partially confined compared with the Dutch selection of dairy herds, leaving less room for seasonal and housing variation in IRCM.

Besides these 3 factors there is a change over time observable in the distribution of mastitis pathogens as it appears from the literature (Myllys et al., 1998; Bradley, 2002). Approximately 2 decades ago, *Staph. aureus* and *Strep. agalactiae* were worldwide the most prevalent mastitis pathogens (Keefe et al., 1997). Nowadays, *E. coli* and *Klebsiella* and other environmental pathogens are emerging pathogens (Myllys et al., 1998; Bradley, 2002; Zadoks and Munoz, 2007). This shift in distribution has been noted by other authors as well (Bradley, 2002; Sol, 2002). Based on our study (Chapter 4) we observed a decline of *Strep. agalactiae* in this study as well, which will be further discussed in following paragraphs. One of the reasons of the shift in mastitis pathogen distribution is obviously the consolidation of dairy farms; larger farms are being built and new farms have generally free-stall barns, instead of tie-stall barns. Additionally, control of mastitis has changed and improved over time, which will also be

discussed in following paragraphs. The initiation of BMSCC bonus programs, as has been done on PEI (Sampson, 2006), will decrease BMSCC even more, and this will result in a shift of the distribution of mastitis pathogens.

8.4 Mastitis control programs

Control of mastitis is based on prevention of new infections and elimination of existing infections (Ruegg, 2003). The "standard mastitis control plan" (5-point mastitis control program) is successful in controlling contagious mastitis pathogens (Neave et al., 1969; Bradley, 2002). However, only 80% of the herds in the random sample of dairy herds were implementing at least 4 of the 5 points (Chapter 4). For example 72% of the herds implemented a blanket dry cow regimen. As discussed in Chapter 4, many of the recommended management practices are already in place on many farms. However, there is also a lot of room for improvement. It is quite surprising that 42% of the producers do not check the milk before they attach the milking unit. Checking of the milk in itself is will not prevent mastitis (Rasmussen et al., 1990; Wagner and Ruegg, 2002), but it will identify underlying mastitis problems, especially if pathogens are involved that usually cause mild clinical mastitis (i.e. only abnormal milk). Segregation of cows during milking is another practice that has been shown to be effective. In our study, it appeared though, that segregation of cows was a cause – effect reversal, because producers that do this practice had a higher overall, *Staph. aureus* and *Strep. dysgalactiae* IRCM than producers that did not do this practice.

The question that arises is, therefore, why do the dairy producers not implement these management practices while the knowledge is in fact available for more than 40

years? An interesting approach is currently implemented by the recently founded National Udder Health Centre in the Netherlands (Lam et al., 2007). They focus on 3 key areas in the forthcoming years: 1) personalize the message; 2) increase producers' frame of reference and give feedback; and 3) use the power of producers' social environment. The results of this project so far have an attractive effect on producers that have not participated so far and seem to get some social pressure due to the success of the program. The feedback and frame of reference that is communicated to all producers might make producers feel they are able to improve their farms and will be recognized for it (Lam et al., 2007). In a recent case-control study by Green et al. (2007) the effect of implementing a mastitis control program was closely followed. After one year in the program there was a significant reductions of 22% in the proportion of cows affected with clinical mastitis on the intervention farms compared with the control farms (Green et al., 2007).

Control programs should therefore be tailor-made for each specific situation, barn type, region, and season of the year. It is therefore important to identify the pathogens involved in a specific herd and keep monitoring them to identify the problem in case a shift may occur, or to identify the area where most efficient success can be obtained. Besides that, season and barn type need to be taken into account when assessing the mastitis situation at the herd level.

The Canadian mastitis control program should not only focus on reducing *Staph. aureus* and information transfer, but should also find ways to motivate producers to implement these practices. The development of a bonus payment program is one example that the majority of dairy producers do appear to support (Sampson, 2006).

8.5 Canadian situation

The distribution of clinical mastitis pathogens in Canada is different per region. The contagious mastitis pathogens *Strep agalactiae* and *Mycoplasma* spp. are present in Canada but in a very low prevalence. Some western provinces might already be *Strep. agalactiae* free. Prince Edward Island could be the first test-case island to become *Strep. agalactiae* free by using the current bulk milk monitoring system to identify the positive herds and closely guide to positive farms to become free. Although we have isolated *Mycoplasma* spp. only from PEI farms, we think that *Mycoplasma* has a low prevalence in other provinces as well. Although frozen storage time and freeze-thaw cycles might affect the isolation of *Mycoplasma* spp., it does not kill it at once (Biddle et al., 2004). If the herd-level prevalence was high, we expect that we would have isolated some *Mycoplasma* from the frozen samples as well. Therefore, we postulate that *Mycoplasma* as a mastitis pathogen is present on some dairy farms in Canada, but it does not appear to be as large a problem as in some areas in the USA (Kirk et al., 1997; González and Wilson, 2003; Fox et al., 2003). Therefore, in Canada, *Mycoplasma* should not be overlooked and diagnostic veterinary laboratories should acquire and maintain knowledge about *Mycoplasma* mastitis and diagnostics. Veterinarians should be aware of the possibility of a *Mycoplasma* outbreak on dairy farms. In contrast, *Staph. aureus* is widely present on Canadian dairy farms. However, a lower prevalence was found in British Columbia which is the only province with a bonus system for low BMSCC. Recently, PEI has initiated a similar bonus system. We suggest that other provinces in Canada should follow the same practice, because financial incentives appear to be a very strong motivator to change mastitis management on farm. A vast

majority of 73% of the Canadian producers do agree that putting a low BMSCC bonus system in place is a good thing and an overwhelming 91% were prepared to change their management if a system was in place (results not shown).

Staph. aureus appears as the most important mastitis pathogen in Canada in clinical mastitis as well. Similar to the bulk milk study we found that *Staph aureus* was most prevalent in Ontario, Québec and the Atlantic provinces. These are the regions where most of the tie-stall barns are. Tie-stall barns and free-stall barns have some distinctly different management practices, as described in Chapter 4. However, with the current knowledge to reduce *Staph. aureus* on a dairy farm it should be possible to reduce both the incidence of *Staph. aureus* IMI and BMSCC, which has been shown to be more or less possible in British Columbia.

8.6 Conclusion

This thesis has provided an estimate of the IRCM on a selection of Canadian dairy farms in all provinces, determined risk factors for IRCM and pathogen-specific IRCM. This thesis also gave some insight in the prevalence of contagious mastitis pathogens at the herd-level in a random sample of Canadian dairy farms, adoption of management practices, the association of management practices with bulk milk prevalence of contagious mastitis pathogens, reported that mastitis-causing *Mycoplasmas* are present in Canada. We showed that season has an effect on all udder health parameters, BMSCC, individual cow SCC, and IRCM. And finally, that quarter SCC fluctuates during and between milking which has consequences for implementing

udder health programs that use SCC to identify cows with IMI. Therefore, the conclusions of this thesis are:

- The mean IRCM of selected Canadian dairy was 22 cases per 100 cow-years (Chapter 2).
- Ontario and Québec have the highest IRCM, mainly of the association of predominating barn type in these provinces (Chapter 2).
- Tie-stall barns had the highest *Staph. aureus*, coagulase-negative staphylococci, and *Strep. uberis* IRCM, whereas free-stall barns had the highest *E. coli* and *Klebsiella* IRCM (Chapter 2).
- The most frequently isolated pathogens from clinical mastitis in Canada are *Staph. aureus*, *E. coli*, *Strep. uberis*, and coagulase-negative staphylococci (Chapter 2).
- Several risk factors were associated with overall and pathogen-specific IRCM (Chapter 3).
- Pathogen-specific risk factors can be quite different, and it is therefore important in mastitis control programs to identify the pathogens that causes problems in a herd (Chapter 3)
- *Staphylococcus aureus* is present in nearly all Canadian dairy farms, whereas *Strep. agalactiae* may be at the brink of extinction in Canada (Chapter 4).
- Reducing *Staph. aureus* prevalence is an important tool to reduce BMSCC (Chapter 4).

- Management on most Canadian dairy farms is good, but there is still room for improvement, where the main problem is how to reach these producers (Chapter 4).
- *Mycoplasma* spp. are present in 2% of the herds in PEI. It is likely that *Mycoplasma* are prevalent at low levels in the rest of Canada (Chapter 4 and 5).
- Agreement between repeated *Staph. aureus* culture from bulk milk samples is moderate. To increase reliability more samples are needed to determine presence of *Staph. aureus* in the bulk milk (Chapter 5).
- Season is associated with BMSCC, IRCM, pathogen-specific IRCM, and individual cow SCC (Chapter 6).
- *Streptococcus uberis* IRCM seems to be associated with pasture, whereas other streptococci and *E. coli* seem to be associated with housing (Chapter 6).
- In quarter samples collected between milkings, SCC is a less reliable indicator of the IMI status than immediately before milking. To be able to make optimal interpretations of SCC tests, whether by laboratory, portable SCC devices, or CMT, veterinarians, researchers, and udder health advisors should take the milk samples immediately before milking (Chapter 7).
- Differential cell ratios did not change much during the time between milking, except in quarters with an elevated SCC (> 200,000 cells/mL), which had a larger proportion of polymorphonuclear leucocytes.

8.7 Future research

In view of the results obtained in this thesis and with special regards to the individual chapters, several aspects of the mastitis situation in Canada should be investigated further. A study of the IRCM as in Chapter 2 has some drawbacks, because firstly, the farms were not randomly selected and do therefore not necessarily represent the national IRCM. Secondly, motivation and correct detection of mastitis by participating producers were perhaps suboptimal. A study with a random selection of herds per province in which farmers are well motivated to take samples of clinical mastitis cases is, although practically difficult, prompted. A recent study in the U.K. has more or less dealt with this problem by selecting farms at random and ask the producers to take samples of only the first 5 cases of mastitis, which is much less of an effort (Bradley et al., 2007).

Because milk samples were frozen and true *Mycoplasma* prevalence is low (Chapter 4), the same set of milk samples should be subjected to a different test method. The best suitable test method for this moment would be a real-time PCR or a blocking ELISA for which recent research have shown some promising results with regards to the sensitivity and specificity (Cai et al., 2005; Ghadersohi et al., 2005). From the same data it was clear that *Strep. agalactiae* is on the brink of extinction in Canadian dairy farms. A new study in the herds that are still affected with *Strep. agalactiae* should determine the source of these infections. Earlier research has shown that cows can be infected with *Strep. agalactiae* of human origin or with low virulent *Strep. agalactiae* (Bramley and Hogben, 1982; Keefe, 1997; Dogan et al., 2005). In the same light, a feasibility study of the commercial use of bulk milk culture should be studied to give

veterinary practices and other udder health advisors additional tools to identify mastitis problems on a farm and a starting point to implement a mastitis control program.

Because the dry period is a time that many new IMI occur (Bradley and Green, 2004), which has also been shown in the studies of Chapters 3 and 4 that dry cow treatment with antibiotics are effective in the reduction of mastitis, new methods of reducing the number of new IMI in that period should be investigated without the use of antibiotics. Increasing public awareness regarding the large amounts of antibiotics used in the dairy industry and the emergence of organic dairying prompt the need to search for alternative methods (Bradley, 2002; Pyorala, 2002).

To increase the understanding of the epidemiology of mastitis pathogens, more research should be initiated using strain typing methods. As has been shown in Chapter 6, *Strep. uberis* IRCM is highest in August and *E. coli* IRCM was only higher in totally confined herds in summer. Additionally, previous research has shown that the classic distinction of environmental and contagious mastitis pathogens is evaporating, certain strains of *Klebsiella* and *Strep. uberis*, for example, which are traditionally called environmental pathogens, do sometimes show contagious properties (Zadoks et al., 2001; Zadoks et al., 2003; Zadoks and Munoz, 2007).

Because a seasonal effect was found in overall and pathogen-specific IRCM in Dutch dairy herds, a similar study should be conducted in Canadian dairy farms involving more farms than the study in Chapter 2. The main reasons for not finding a seasonal effect in the Canadian study (Chapter 2) were that, compared with the Dutch study, fewer farms were involved in the study, more farms were totally confined, and about half of the Canadian farms had tie-stall barns, whereas in the Dutch study only free-stall barns were involved.

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APPENDIX 1

Canadian Bovine Mastitis
Research Network
Réseau canadien de recherche
sur la mammite bovine

MASTITIS MANAGEMENT

questionnaire

Farmname:	.....
Contact person:	.....
Telephone:	(.....) ..... (home)
	(.....) ..... (cell phone)
Date:	.....
Interviewer:	.....

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1. General questions about the farm and mastitis

1.1. How many cows do you currently have?

(a) ..... lactating cows

(b) ..... dry cows

(c) .....bred heifers

1.2. Type of housing for the lactating cows, dry cows and bred heifers (☒ **all** that apply):

(a) **Lactating cows:**	(b) **Dry cows:**	(c) **Bred heifers:**
☐ *Tie-stall*	☐ *Tie-stall*	☐ *Tie-stall*
☐ *Free-stall*	☐ *Free-stall*	☐ *Free-stall*
☐ *Manure / straw pack*	☐ *Manure / straw pack*	☐ *Manure / straw pack*
☐ *Other: (please specify)*	☐ *Other: (please specify)*	☐ *Other: (please specify)*
.....	.....	.....

(d) Are bred heifers and dry cows housed together?

☐ *Yes* ☐ *No*

(e) *If you have free-stall or straw-pack barn*, how many bunk spaces do the cows have?

Lactating cows ..... *spaces*

Dry cows ..... *spaces*

Bred heifers ..... *spaces*

(f) *If you have a free-stall barn,* how many stalls do the cows have?

Lactating cows ..... *stalls*

Dry cows ..... *stalls*

Bred heifers ..... *stalls*

1.3. (a) Do the **lactating cows** go to pasture in the Summer?

☐ *No, they stay in the barns all year round*

☐ *No, but they only have access to an exercise yard (less than 5 acres / 100 cows)*

☐ *Yes, they go on pasture from the month ..... until .....*

(b) In the pasture season, are your cows

☐ *Outside day and night*

☐ *Inside only at night*

☐ *Other: (please specify) .....*

1.4. Do you have set goals for udder health performance written down on paper?

☐ *Yes* ☐ *No*

1.4a. Do you have your milking procedures written down on paper?

☐ *Yes* ☐ *No*

1.5. (a) What was your average Bulk Milk Somatic Cell Count last year ? ..... *cells/ml*

(b) When is your BMSCC highest? (☒ **all** that apply)

- ☐ *Winter*
- ☐ *Spring*
- ☐ *Summer*
- ☐ *Autumn*
- ☐ *Same all year*

---

2. Milking procedures

2.1. How often do you milk?

☐ *Twice a day* ☐ *Three times a day* ☐ *Other: (please specify)* .....

2.2. What are your milking times approximately?

1st milking starts at (time) _____ *and ends at* _____

2nd milking starts at (time) _____ *and ends at* _____

3rd milking starts at (time) _____ *and ends at* _____

2.3. How many different people have been milking the cows in the last week (include temporary / relief milkers)?

..... *female milkers*

..... *male milkers*

2.3a. When do you train your milking employees?

- ☐ *Never*
- ☐ *Always just after I have hired them*
- ☐ *Whenever I feel it is needed*
- ☐ *Other: (please specify)* .....

2.4. (a) Do you do **any** udder preparation before you attach the milking unit?

☐ *Yes* ☐ *No (please proceed to question 2.8)*

(b) Do you use water to clean the teats?

☐ *Yes* ☐ *No (please proceed to question 2.5)*

(c) *If you do use water*, what do you use to dry the teat

- ☐ *Nothing, I do not dry the teats*
- ☐ *Cloth or towel*
- ☐ *Paper towel or newspaper*
- ☐ *Other: (please specify)* .....

(d) How many cows do you dry with one towel / cloth?

..... *cows*

2.5. (a) Do you use a **pre-dip** or **spray**?

☐ *Yes* ☐ *No (please proceed to question 2.6)*

(b) What brand do you use?

☐ *Della PreTech® (DeLaval)*
☐ *Theratech® Pre & Post (WestfaliaSurge)*

☐ *Other: (please specify)* .....

(c) How do you wipe the teats after dipping or spraying?

☐ *Nothing, I do not wipe the teats after dipping / spraying*
☐ *Cloth or towel*
☐ *Paper towel or newspaper*
☐ *Other: (please specify)* .....

(d) How many cows do you wipe with one towel / cloth?

..... *cows*

2.6. (a) If you **do not** predip or spray, do you **wipe** the teats?

☐ *Yes* ☐ *No (please proceed to question 2.7)*

(b) What do you use for wiping the teats?

☐ *Commercially available "wet" disinfecting towel, similar to ReadyWipe®*
☐ *Dry towel or cloth*
☐ *Sponge*
☐ *Cloths or towels soaked in water (with or without disinfectant)*
☐ *Other: (please specify)* .....

(c) How many cows do you wipe with one towel / cloth?

..... *cows*

2.7. (a) Do you **strip** teats before milking?

☐ *Yes*
☐ *No*
☐ *Only when I have problems with mastitis*

(b) If you strip before milking, when do you do that? (☒ **all** that apply)

☐ *Every cow at every milking*
☐ *Only mastitis suspicious cows*
☐ *High SCC cows*
☐ *Cows with clinical mastitis*
☐ *Other: (please specify)* .....

2.8. Do you apply **post-dip** (or spray)?

(a) ☐ *Yes* ☐ *No (please proceed to question 2.9)*

(b) What do you use?

☐ *Dipping cups*
☐ *Manual sprayer*
☐ *Automated sprayer*
☐ *Other: (please specify)* .....

(c) What brand of post-dip do you use?

- ☐ *Della One® (DeLaval)*
- ☐ *Della Soft® (DeLaval)*
- ☐ *Teat-Kote® (WestfaliaSurge)*
- ☐ *Bovi-Kote® (Bou-Matic)*
- ☐ *Emerald® (ABS Global)*
- ☐ *Protek® (Ecolab)*
- ☐ *Mastimin 50 Dripless®*
- ☐ *Uddergold® (Ecolab)*
- ☐ *Theratec Pre & Post® (WestfaliaSurge)*
- ☐ *Other: (please specify) .....*

(d) Do you post-dip or spray all year round?

- ☐ *Yes*
- ☐ *No, I do not post-dip or spray from ..... to .....*

2.9. Does your equipment have automated takeoffs?

(a) ☐ *Yes (please proceed to question 2.9c)* ☐ *No*

(b) Do you shut the vacuum off before cup removal?

☐ *Yes* ☐ No

(c) At what flow does your equipment take the units off?

- ☐ *I don't know*
- ☐ *At ..... kg / min*

2.10. Do you and your milkers wear latex (or similar) gloves during milking?

☐ *Yes* ☐ *Sometimes* ☐ *No*

2.11. How many cows do you have to restrain at milking?

..... *cows*

2.12. (a1) Do you milk cows with **high somatic cell count** cows last and/or with a separate unit?

☐ *Yes* ☐ *No*

(a2) Do you clean the milking unit after you have milked a high somatic cell count cow?

- ☐ *Yes, after every cow*
- ☐ *Sometimes*
- ☐ *No*

(b1) Do you milk ***Staphylococcus aureus* infected** cows last and/or with a separate unit?

☐ *Yes* ☐ *No*

(b2) Do you clean the milking unit after you have milked a *Staphylococcus aureus* infected cow?

- ☐ *Yes, after every cow*
- ☐ *Sometimes*
- ☐ *No*

(c1) Do you milk cows with **mastitis** last and/or with a separate unit?

☐ *Yes* ☐ *No*

(c2) Do you clean the milking unit after you have milked a cow with mastitis?

- ☐ *Yes, after every cow*
- ☐ *Sometimes*
- ☐ *No*

2.13. What do you do to prevent the cows from lying down after milking?

- ☐ *Nothing*
- ☐ *Provide fresh feed*
- ☐ *Lock them in the head locks*
- ☐ *Let them stand in a waiting area*
- ☐ *Other: (please specify) .....*

2.14. How many days after calving do you put the milk of the fresh cow in the tank?

..... *days*

---

3. Management of clinical cases of mastitis

3.1. How many cases of mastitis do you think you have per month?

Approximately ..... cases / month

3.2. Do you consider blood in the milk as mastitis?

☐ *Yes* ☐ *No*

3.3. Do you consider abnormal milk right after calving as mastitis?

☐ *Yes* ☐ *No*

3.4. Do you agree with the following statements?

	Disagree	*Somewhat disagree*	*Neutral*	*Somewhat agree*	*Agree*
(a) When present, clinical mastitis is easy to detect	1☐	2☐	3☐	4☐	5☐
(b) I am concerned about the costs of clinical mastitis	1☐	2☐	3☐	4☐	5☐
(c) Culturing of clinical mastitis milk samples is useless	1☐	2☐	3☐	4☐	5☐
(d) I always make sure I finish the treatment (as recommended)	1☐	2☐	3☐	4☐	5☐
(e) I think that antibiotics nowadays are not as effective as before	1☐	2☐	3☐	4☐	5☐
(f) It is often necessary to change antibiotics during treatment	1☐	2☐	3☐	4☐	5☐

3.5. How is clinical mastitis commonly seen or detected on your farm?

	Rarely		*Neutral*		*Very often*
(a) Abnormal milk	1☐	2☐	3☐	4☐	5☐
(b) Abnormal udder	1☐	2☐	3☐	4☐	5☐
(c) Abnormal kicking during milking	1☐	2☐	3☐	4☐	5☐
(d) Sick cow	1☐	2☐	3☐	4☐	5☐
(e) By using a (automated) conductivity meter	1☐	2☐	3☐	4☐	5☐
(f) Other: (please specify)	1☐	2☐	3☐	4☐	5☐

3.6. Do you treat all cases of mastitis with antibiotics?

- ☐ *All cases*
- ☐ *Some cases, approximately ..... % (please specify)*
- ☐ *None*

3.7. Do you disinfect the teat with an alcohol swab before infusion?

- ☐ *Yes*
- ☐ *Sometimes*
- ☐ *No*

3.8. Do you use full (longtip) or partial (shorttip) insertion?

☐ *Full*
☐ *Partial*

3.9. How many antibiotic treatments do you apply to a cow with mastitis as **a minimum**?

Minimum of ..... treatments

3.10. How many treatments do you apply to a cow as **a maximum** if she does not clear up?

Maximum of ..... treatments

3.11. How frequently do you milk out a cow with clinical mastitis?

☐ *Only during normal milking*
☐ *3 – 4 times a day*
☐ *more than 4 times a day*
☐ *Other: (please specify) .....*

3.12. In this study, could you give us an idea of how many clinical mastitis cases you might have forgotter or simply missed to take a sample?

*I did **not** sample approximately ..... cases (please specify)*

3.13. How much do you think a case of clinical mastitis costs on average?

A case of clinical mastitis costs approx. $..... (please specify)

3.14. How do you mark or remember a cow that has been treated? (☒ **all** that apply)

☐ *The cow's name or ID on a white board or chalk board*
☐ *Keep her separate*
☐ *Apply (colored) leg bands*
☐ *Color mark (leg, back, udder, tail, etc)*
☐ *Other: (please specify) .....*

3.15. (a) Do you vaccinate your cows against mastitis?

☐ *All cows*
☐ *Most of them (≥ 50%)*
☐ *Some (<50%)*
☐ *None (please proceed to question 3.16)*

(b) When do you vaccinate?

☐ *At dry-off*
☐ *At precalving*
☐ *Early lactation (0-100 DIM)*
☐ *At midlactation (101-200 DIM)*
☐ *Other: (please specify) .....*

3.16. How many cows did you need to cull due to mastitis in the last year?

..... *cows*

3.17. Some farmers have drawn up a *farm-specific treatment plan* together with their veterinarian, based on sensitivity of bacteria found on their farm and farm specific problems.

(a) Do you have a *farm-specific treatment plan* written on paper or on the computer?
☐ *Yes* ☐ *No*

(b) Do you think that a *farm-specific treatment plan* can be useful?
☐ *Yes* ☐ Maybe ☐ *No*

(c) Would you be interested in drawing up *a farm-specific treatment plan* together with your veterinarian?

No	*Probably not*	*Neutral*	*Maybe yes*	*Yes*
1☐	2☐	3☐	4☐	5☐

---

4. Dry cow management

4.1. (a) What proportion of cows are dry-cow treated with antibiotics at the end of lactation?

Approximately ..... %

(b) Which products do you use? (☒ **all** that apply)
☐ *Dryclox®*
☐ *Cefadry®*
☐ *Novodry®*
☐ *Other: (please specify)* .....

4.2. Do you disinfect the teat with an alcohol swab before infusion?
☐ *Yes*
☐ *Sometimes*
☐ *No*

4.3. Do you use full (longtip) or partial (shorttip) insertion?
☐ *Full*
☐ *Partial*

4.4. (a) Do you use *Orbeseal®* at dry off?
☐ *Yes* ☐ *No (please proceed to question 4.5)*

(b) What proportion of cows are dry-cow treated with *Orbeseal®*?

Approximately ..... %

(c) Do you use *Orbeseal®* in combination with antibiotics?
☐ *Yes, always in combination with antibiotics*
☐ *Sometimes*
☐ *No, I always use Orbeseal® alone*

4.5. Do you reduce the milking frequency in the week before drying off?
☐ *Yes* ☐ *No*

4.6. Do you check the **dry cows** regularly for visible signs of clinical mastitis?

☐ *No, never*
☐ *Yes, every ..... day(s) (please specify the frequency)*

4.7. Do you check the **bred heifers** regularly for visible signs of clinical mastitis?

☐ *No, never*
☐ *Yes, every ..... day(s) (please specify the frequency)*

4.8. What is your average dry period?

..... *days*

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5. High somatic cell count cows (subclinical mastitis)

5.1. At what level of somatic cell counts do you consider a cow a **high** somatic cell count cow?

Cows with a SCC of .....cells/ml and higher

5.2. Do you agree with the following statements?

	Disagree		*Neutral*		*Agree*
(a) High SCC cows are easy to discover during milking	1☐	2☐	3☐	4☐	5☐
(b) I am concerned about the costs of cows with high SCC	1☐	2☐	3☐	4☐	5☐
(c) Culturing milk samples of cows with high SCC is generally useless	1☐	2☐	3☐	4☐	5☐

5.3. The most important ways to discover high SCC cows are: (☒ **all** that apply)

☐ *Individual SCC on the DHI data*
☐ *Observe the cow and her udder*
☐ *Automated testing (conductivity)*
☐ *By using CMT (California Mastitis Test)*
☐ *Other: (please specify) .....*

5.4. How many cows did you need to cull due to high SCC in the last year?

..... *cows*

5.5. Do you take milk samples from cows with **high SCC** for bacterial culture?

☐ *All cows*
☐ *Most of them (≥ 50%)*
☐ *Some (<50%)*
☐ *None*

---

6. Milking equipment

6.1. The vacuum level during milking is:

☐ ..... *kPa or ..... psi*
☐ *I don't know exactly*

6.2. If you have a **tie-stall**, what type of milking system do you have?

- ☐ *Pipeline*
- ☐ *Buckets*

6.3. *If you* **do not** *have a tie-stall,*

(a) What type of milking parlor do you have?

- ☐ *Herring bone*
- ☐ *Side by side (parallel)*
- ☐ *Tandem (Side-opening)*
- ☐ *Automated Milking System (Robot)*
- ☐ *Rotary*
- ☐ *Other: (please specify)* .....

(b) What type of milk line do you have?

- ☐ High-level
- ☐ Low-level
- ☐ *Other: (please specify)* .....

(c) Do the cows have access to water in the waiting areas before milking?

☐ *Yes* ☐ *No*

6.4. How many units does your milking system have (tie-stall or parlor)?

..... *milking units*

6.5. How often is the functioning of your milking equipment checked and analyzed by the **equipment dealer**?

- ☐ *Twice or more times per year*
- ☐ *Once a year*
- ☐ *Less than once a year*
- ☐ *Never*

6.6. How often is the functioning of your milking equipment checked and analyzed by **an independent technician**?

- ☐ *Twice or more times per year*
- ☐ *Once a year*
- ☐ *Less than once a year*
- ☐ *Never*

6.7. How often do you check the vacuum?

- ☐ *Never*
- ☐ *Only if I have mastitis problems*
- ☐ *Once a month*
- ☐ *Once a week*
- ☐ *Almost every day*
- ☐ *Other: (please specify)* .....

6.8. Did you have your barn and / or milking equipment checked for stray-voltage in the last 2 years?

☐ *Yes* ☐ *No*

7. Cow comfort and hygiene

7.1. What material does the stall base consist of? (☒ **all** that apply)

(a) **Lactating cows:**	(b) **Dry cows:**	(c) **Bred heifers:**
☐ *Concrete*	☐ *Concrete*	☐ *Concrete*
☐ *Mattres*	☐ *Mattres*	☐ *Mattres*
☐ *Rubber mat*	☐ *Rubber mat*	☐ *Rubber mat*
☐ *Clay*	☐ *Clay*	☐ *Clay*
☐ *Other: (please specify)*	☐ *Other: (please specify)*	☐ *Other: (please specify)*
.....	.....	.....

7.2. What material do you use as bedding? (☒ **all** that apply)

(a) **Lactating cows:**	(b) **Dry cows:**	(c) **Bred heifers:**
☐ *None*	☐ *None*	☐ *None*
☐ *Sawdust*	☐ *Sawdust*	☐ *Sawdust*
☐ *Shavings*	☐ *Shavings*	☐ *Shavings*
☐ *Sand*	☐ *Sand*	☐ *Sand*
☐ *Straw*	☐ *Straw*	☐ *Straw*
☐ *Other: (please specify)*	☐ *Other: (please specify)*	☐ *Other: (please specify)*
.....	.....	.....

7.3. How often do you **clean out the manure** in the stalls? (for example scraping the back 1/2 of the stalls out) (☒ **all** that apply)

(a) **Lactating cows:**	(b) **Dry cows:**	(c) **Bred heifers:**
☐ *Twice a day or more*	☐ *Twice a day or more*	☐ *Twice a day or more*
☐ *Once a day*	☐ *Once a day*	☐ *Once a day*
☐ *Once every two days*	☐ *Once every two days*	☐ *Once every two days*
☐ *Other: (please specify)*	☐ *Other: (please specify)*	☐ *Other: (please specify)*
.....	.....	.....

7.4. How often do you **change the bedding** in the stalls (☒ **all** that apply)?

(a) **Lactating cows:**	(b) **Dry cows:**	(c) **Bred heifers:**
☐ *Once a day*	☐ *Once a day*	☐ *Once a day*
☐ *Once every two days*	☐ *Once every two days*	☐ *Once every two days*
☐ *Twice a week*	☐ *Twice a week*	☐ *Twice a week*
☐ *Other: (please specify)*	☐ *Other: (please specify)*	☐ *Other: (please specify)*
.....	.....	.....

7.5. *If you have a free-stall,*

(a) how are the alleys cleaned (☒ **all** that apply)?

- ☐ *Manual*
- ☐ *Automated*
- ☐ *Skid-steer or tractor*
- ☐ *Other: (please specify)* .....

(b) How often are the alleys scraped per day?

..... *times / day*

7.6. Do you clip or flame udders and how often?

☐ *No*
☐ *Clip,* ..... *times / year*
☐ *Flame,* ... . *times / year*

7.7. Do you clip or dock tails?

☐ *No*
☐ *Clip,* ..... *times / year*
☐ *Dock*

7.8. (a) Do you have a **maternity pen / calving stall**?

☐ *Yes* ☐ *No (please proceed to question 8.1)*

(b) Are the sick cows housed in the same pen?

☐ *Yes* ☐ *No*

(c) What kind of bedding material do you use in the maternity pen?

☐ *None*
☐ *Sawdust*
☐ *Shavings*
☐ *Sand*
☐ *Straw*
☐ *Other: (please specify)* .....

(d) How often is the bedding replaced by clean bedding?

☐ *After every calving*
☐ *Other: (please specify)* .....

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8. Biosecurity and prevention

8.1. Do visitors to your barns have to disinfect their boots or shoes?

☐ *Yes* ☐ *No*

8.2. Do visitors to your barn have to wear protective clothing, **provided by you** (e.g. boots, overalls)

☐ *Yes* ☐ *No*

8.3. (a) How many heifers and cows on your farm are purchased animals?

..... *heifers purchased* ..... *cows purchased*

(b) If you purchase cows, do you request information on (Somatic) Cell Counts prior to purchase?

☐ *Always request SCC information*
☐ *Usually (≥50%)*
☐ *Sometimes (<50%)*
☐ *Never*

8.4. (a) Do you treat heifers with antibiotics prior to calving as a mastitis prevention measure?

- ☐ *Yes*
- ☐ *Sometimes*
- ☐ *No (please proceed to question 8.5)*

(b) How do you do it?

- ☐ *In the muscle (in the neck, rump, etc)*
- ☐ *In the udder with Dry Cow treatment*
- ☐ *In the udder with Lactating Cow treatment*
- ☐ *Other: (please specify) .....*

8.5. Do you agree with the following statements?

	Disagree			*Neutral*		*Agree*
(a) I always monitor my BMSCC very closely	1☐	2☐	3☐	4☐	5☐	
(b) If I want to, I can reduce my BMSCC	1☐	2☐	3☐	4☐	5☐	
(c) I think an analysis of individual cow SCCs is very important	1☐	2☐	3☐	4☐	5☐	
(d) I would like to reduce the amount of cows with mastitis	1☐	2☐	3☐	4☐	5☐	
(e) I generally know what causes the increase of cases of mastitis on my farm	1☐	2☐	3☐	4☐	5☐	
(f) Generally you cannot influence causes of mastitis	1☐	2☐	3☐	4☐	5☐	
(g) Bad luck is an important factor in a mastitis outbreak	1☐	2☐	3☐	4☐	5☐	

8.6. During a ***Staphylococcus aureus*** outbreak it is important to:

- ☐ *Keep the stalls very clean, because this bacteria spreads itself mainly through manure, bedding and the environment of the cow.*
- ☐ *Pay extra attention to hygiene during milking and milking procedures.*
- ☐ *I don't know*

8.7. During a ***E. coli*** outbreak it is important to:

- ☐ *Keep the stalls very clean, because this bacteria spreads itself mainly through manure, bedding and the environment of the cow.*
- ☐ *Pay extra attention to hygiene during milking and milking procedures.*
- ☐ *I don't know*

8.8. Do you agree with the following statements (the questions about submitting samples should be answered regarding the **normal** situation, not the sampling for this study)?

	Disagree			*Neutral*		*Agree*
(a) If I have sudden increase in cases of mastitis, I would like to know the bacteria that causes it ...	1☐	2☐	3☐	4☐	5☐	
(b) I think bacteriologic testing is too expensive	1☐	2☐	3☐	4☐	5☐	
(c) I think it takes too long before I receive the laboratory results from submitted samples	1☐	2☐	3☐	4☐	5☐	
(d) Interpreting the laboratory results is difficult	1☐	2☐	3☐	4☐	5☐	
(e) Bacteriologic testing / culturing is important because it determines the direction of the treatment	1☐	2☐	3☐	4☐	5☐	
(f) Treatment and prevention of mastitis is important on my farm	1☐	2☐	3☐	4☐	5☐	
(g) I know enough about mastitis to keep me out of trouble	1☐	2☐	3☐	4☐	5☐	
(h) I should do more about mastitis prevention	1☐	2☐	3☐	4☐	5☐	
(i) I do not have enough time for mastitis prevention	1☐	2☐	3☐	4☐	5☐	

8.9. Do you use a computer for keeping records of your cows?
☐ *Yes* ☐ *No*

8.10. In which record system do you keep records of mastitis cases? (☒ **all** that apply)
☐ None
☐ White board, chalk board or similar
☐ Cow cards
☐ Breeding wheel
☐ A 21-day calendar
☐ Cow diary
☐ Computer
☐ Other: *(please specify)* .....

8.11. Which data do you record of each mastitis case? (☒ **all** that apply)

Cow name or number	☐ *Yes*	☐ *No*
Which quarter is affected	☐ *Yes*	☐ *No*
Severity	☐ *Yes*	☐ *No*
Date of onset	☐ *Yes*	☐ *No*
Date of last treatment	☐ *Yes*	☐ *No*
Type of treatment	☐ *Yes*	☐ *No*
Number of treatments	☐ *Yes*	☐ *No*
Date return in bulk tank	☐ *Yes*	☐ *No*
Type of bacteria after culture	☐ *Yes*	☐ *No*

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9. Nutrition

9.1. Is the ration you feed to the cows a TMR (Total Mixed Ration)?
☐ *Yes* ☐ *No*

9.2. How often are the cows' rations balanced based on forage analyses?
☐ *Three or more times per year*
☐ *Twice a year*
☐ *Once a year*
☐ *Less than once a year*
☐ *Never*

9.3. Do you feed to your lactating cows?

Corn-silage	☐ *Yes*	☐ *No*
Potatoes	☐ *Yes*	☐ *No*
Sugar beet pulp	☐ *Yes*	☐ *No*

9.4. Do you feed the left-overs of the lactating cows to the dry cows?
☐ *Yes* ☐ *No*

9.5. In which period of the lactation do the cows get their highest feed energy levels?

Approximately from ..... DIM to ..... DIM

9.6. How many days before drying off do you reduce **feed energy levels**?
☐ *No feed or energy reduction*
☐ ..... *days before dry-off (please fill in the number of days)*

9.7. How many days before drying off do you reduce **water intake**?

☐ *No water intake reduction*

☐ ..... *days before dry-off (please fill in the number of days)*

9.8. (a) Do you use mineral and trace-element additives *in the ration*?

☐ *Yes* ☐ *No (please proceed to question 9.9)*

(b) What kind of additives do you give?

(I) **Lactating cows:**	(II) **Dry cows:**	(III) **Bred heifers:**
☐ *Commercial mix*	☐ *Commercial mix*	☐ *Commercial mix*
☐ *Multivitamin preparations*	☐ *Multivitamin preparations*	☐ *Multivitamin preparations*
☐ *Vitamin E*	☐ *Vitamin E*	☐ *Vitamin E*
☐ *Selenium (Se)*	☐ *Selenium (Se)*	☐ *Selenium (Se)*
☐ *Cupper (Cu)*	☐ *Cupper (Cu)*	☐ *Cupper (Cu)*
☐ *Magnesium (Mg)*	☐ *Magnesium (Mg)*	☐ *Magnesium (Mg)*
☐ *Sodium (Na)*	☐ *Sodium (Na)*	☐ *Sodium (Na)*
☐ *Potassium (K)*	☐ *Potassium (K)*	☐ *Potassium (K)*
☐ *Calcium (Ca)*	☐ *Calcium (Ca)*	☐ *Calcium (Ca)*
☐ *Rumensin®*	☐ *Rumensin®*	☐ *Rumensin®*
☐ *Niacin®*	☐ *Niacin®*	☐ *Niacin®*
☐ *Yeast*	☐ *Yeast*	☐ *Yeast*
☐ *Kelp or seaweed*	☐ *Kelp or seaweed*	☐ *Kelp or seaweed*
☐ *Zinpro®*	☐ *Zinpro®*	☐ *Zinpro®*
☐ *Other: (please specify)*	☐ *Other: (please specify)*	☐ *Other: (please specify)*
.....	.....	.....

9.9 (a) Do you *inject* cows with minerals / vitamins / trace-elements?

☐ *Yes* ☐ *No (please proceed to question 9.10)*

(b) Which minerals / vitamins / trace-elements do you inject?

☐ *Vitamin B (any)*

☐ *Vitamin D (any)*

☐ *Vitamin E / Selenium*

☐ *Multivitamin preparations*

☐ *Other: (please specify)* .....

9.10 In formulating the cow's rations, can you indicate the role of each of the following persons:

	Not important			*Neutral*		*Very important*
(a) *Independent nutritionist*	1☐	2☐	3☐	4☐	5☐	
(b) *Feed company representative*	1☐	2☐	3☐	4☐	5☐	
(c) *Veterinarian*	1☐	2☐	3☐	4☐	5☐	
(d) *DHI representative (or equivalent Canwest DHI, PATLQ or ADLIC)*	1☐	2☐	3☐	4☐	5☐	
(e) *Other: (please specify)*	1☐	2☐	3☐	4☐	5☐	

9.11. What is the water source for the cows?

- ☐ *A dug well*
- ☐ *A drilled well*
- ☐ *Surface water (creek, river, lake, pond, etc)*
- ☐ *Central (municipal) water*
- ☐ *Other: (please specify) .....*

9.12. Has a water analysis been made in the last 2 years?

(a) Tested for bacteria ..... ☐ *Yes* ☐ *No*

(b) Tested for mineral content ..... ☐ *Yes* ☐ *No*

(c) If you had it tested for bacteria, were there any problems with the water quality?

☐ *Yes* ☐ *No*

---

10. Mastitis plan review and communication

10.1. Who is important in reviewing your mastitis data and / or plan with you?

	Not important				*Very important*
(a) Veterinarian	1☐	2☐	3☐	4☐	5☐
(b) DHI representative (or equivalent Canwest DHI, PATLQ or ADLIC)	1☐	2☐	3☐	4☐	5☐
(c) Nutritionist	1☐	2☐	3☐	4☐	5☐
(d) Milking equipment representative	1☐	2☐	3☐	4☐	5☐
(e) Other farmers	1☐	2☐	3☐	4☐	5☐
(f) Family member(s)	1☐	2☐	3☐	4☐	5☐
(g) Other: (please specify)	1☐	2☐	3☐	4☐	5☐

10.2. (a) Do you check your DHI data the same day that you receive it?

☐ *Yes* ☐ *No*

(b) How often do you sit down and review your mastitis data? (☒ **all** that apply)

- ☐ *Once a week*
- ☐ *Twice a month*
- ☐ *Once a month*
- ☐ *Twice a year*
- ☐ *Only when my bulk tank somatic cell counts exceeds 200,000 cells/ml*
- ☐ *Only when my bulk tank somatic cell counts exceeds 400,000 cells/ml*
- ☐ *Other: (please specify) .....*

10.3. If your veterinarian organized a fee-based course about mastitis prevention, consisting of 2 day-parts, would you participate?

- ☐ *No*
- ☐ *Yes, and I would pay a maximum of $.....*

10.4. How much time do you spend on reading literature related to dairy health management per week?

I would spend approx. ..... hours

10.5. Which magazine do you prefer to read? (☒ **all** that apply)

- ☐ *The Milk Producer*
- ☐ *Le producteur de lait québécois*
- ☐ *Western Dairy Digest*
- ☐ *Hoard's Dairyman*
- ☐ *Other: (please specify) .....*

10.6. Do you agree with the following statements?

	Disagree		*Neutral*		*Agree*
(a) I would certainly read articles about mastitis in my dairy magazines	1☐	2☐	3☐	4☐	5☐
(b) I would like to see more herd management articles in my dairy magazines	1☐	2☐	3☐	4☐	5☐

10.7. If you would like to know more about mastitis prevention, which **three (3)** methods would be **most** important to you:

- ☐ *Articles in dairy magazines*
- ☐ *Special website on the internet*
- ☐ *Discussions with other farmers*
- ☐ *A symposium with a mastitis expert*
- ☐ *Advise from my veterinarian*
- ☐ *Reading a mastitis manual or handbook*
- ☐ *A free help desk*
- ☐ *Video course*
- ☐ *CD-rom self help program*
- ☐ *A mastitis expert visiting my farm on a regular basis*
- ☐ *A mastitis course*

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11. More general questions

11.1. At which **Bulk Milk Somatic Cell Count** level do you think you have a mastitis problem?

At .....,000 cells/ml

11.2. At what incidence of **clinical mastitis** do you think you have a mastitis problem ("clinical mastitis"is here defined as "visible abnormality of the milk and / or the udder")?

*When the number of clinical mastitis exceeds ..... cases per **month***

11.3. At what incidence of **new high somatic cell count cows** (all cows that have SCC greater than 200,000 cells/ml) do you think you have a mastitis problem?

*When the number of new high somatic cell count cows exceeds ..... cases per **month***

11.4. Have you ever had a mastitis problem as described above?

☐ *Yes* ☐ *No*

11.5. Have you had problems with mastitis in the last 2 years?

A few		*Average*		*A lot*
1☐	2☐	3☐	4☐	5☐

11.6. Do you agree with the following statements?

	Disagree			Neutral	Agree
(a) In general I manage mastitis well on my farm	1☐	2☐	3☐	4☐	5☐
(b) Mastitis is a difficult disease for me	1☐	2☐	3☐	4☐	5☐
(c) Every case of mastitis bothers me a lot	1☐	2☐	3☐	4☐	5☐
(d) Every case of mastitis gives a lot of extra work	1☐	2☐	3☐	4☐	5☐

11.7. What is to you the most bothersome aspect of mastitis? (only **one** answer please)

- ☐ *Disturbance of my milking routine*
- ☐ *Financial consequences*
- ☐ *Extra labor*
- ☐ *Other: (please specify)* .....

11.8. Do you agree with the following statements?

	Disagree			Neutral	Agree
(a) I worry about mastitis quite often	1☐	2☐	3☐	4☐	5☐
(b) I think I handle mastitis prevention and treatment the right way	1☐	2☐	3☐	4☐	5☐
(c) As long as mastitis problems are not getting too serious, I don't change anything	1☐	2☐	3☐	4☐	5☐
(d) I changed my management in the last five years, because of mastitis problems	1☐	2☐	3☐	4☐	5☐
(e) Udder health is an important aspect in bull selection	1☐	2☐	3☐	4☐	5☐

11.9. How would you qualify your knowledge about the following subjects:

	Insufficient	Less than sufficient	Sufficient	Good	Excellent
(a) Influence of nutrition on mastitis	1☐	2☐	3☐	4☐	5☐
(b) Milking equipment	1☐	2☐	3☐	4☐	5☐
(c) Milking procedures	1☐	2☐	3☐	4☐	5☐
(d) Barn type and barn hygiene	1☐	2☐	3☐	4☐	5☐
(e) The proper use of DHI records for mastitis management	1☐	2☐	3☐	4☐	5☐
(f) Type of bacteria and bacterial culturing	1☐	2☐	3☐	4☐	5☐
(g) Treatment of clinical mastitis	1☐	2☐	3☐	4☐	5☐
(h) Treatment of subclinical mastitis	1☐	2☐	3☐	4☐	5☐
(i) Use of medications for mastitis	1☐	2☐	3☐	4☐	5☐
(j) Buying and culling policy of animals	1☐	2☐	3☐	4☐	5☐

12.1. How much interest do you have for the following aspects of dairy farming:

	No interest			Neutral	A lot of Interest
(a) Pasture management	1☐	2☐	3☐	4☐	5☐
(b) Breeding	1☐	2☐	3☐	4☐	5☐
(c) Economics and financial management	1☐	2☐	3☐	4☐	5☐
(d) Management of minerals and trace elements	1☐	2☐	3☐	4☐	5☐
(e) Nutrition	1☐	2☐	3☐	4☐	5☐
(f) Machinery	1☐	2☐	3☐	4☐	5☐
(g) Animal health	1☐	2☐	3☐	4☐	5☐
(h) Calf / young stock growing	1☐	2☐	3☐	4☐	5☐
(i) Milking	1☐	2☐	3☐	4☐	5☐
(j) Labor planning	1☐	2☐	3☐	4☐	5☐
(k) Other income than dairy	1☐	2☐	3☐	4☐	5☐

12.2. How important are these aims on your farm?

	Not important			*Neutral*		*Very important*
(a) High milk production per cow	1☐	2☐	3☐	4☐	5☐	
(b) As many cows as possible per acre	1☐	2☐	3☐	4☐	5☐	
(c) As many cows as possible per person	1☐	2☐	3☐	4☐	5☐	
(d) Expand the farm with more land	1☐	2☐	3☐	4☐	5☐	
(e) Expand the farm with more quota	1☐	2☐	3☐	4☐	5☐	
(f) Keep the management simple	1☐	2☐	3☐	4☐	5☐	
(g) As low as possible debt	1☐	2☐	3☐	4☐	5☐	
(h) Try to get as high returns as possible	1☐	2☐	3☐	4☐	5☐	
(i) Get income other than dairy	1☐	2☐	3☐	4☐	5☐	
(j) Plan on an easy succession of my son or daughter	1☐	2☐	3☐	4☐	5☐	

12.3. (a) Do you think a premium should be paid for low somatic cell count milk?

☐ *Yes* ☐ *No* ☐ *No opinion*

(b) Would you change your management style in order to receive that premium?

☐ *Yes* ☐ *Maybe* ☐ *No*

12.4. Do you have any other comments?

☐ *This questionnaire was difficult*
☐ *This questionnaire was too long*
☐ *Other (please use the box for additional comments)*

Thank you for your time !

APPENDIX 2

Réseau canadien de recherche
sur la mammite bovine
Canadian Bovine Mastitis
Research Network

Étude canadienne sur la régie de la mammite

Questionnaire

Nom de la ferme:	.....
Personne contact:	.....
Téléphone:	(.....) ..... (maison)
	(.....) ..... (cellulaire)
Date:	.....
Enquêteur:	.....

Pour toutes questions ou commentaires, veuillez contacter:

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Producteurs laitiers du Canada
Réseau canadien de recherche sur la mammite bovine
Conseil de recherches en sciences naturelles et en génie
Atlantic Veterinary College

1. Questions générales

1.1. Combien de vaches avez-vous actuellement? *(a) ..... vaches en lactation*
(b) ..... vaches taries
(c) .....taures saillies

1.2. Type de logement pour les vaches en lactation, les vaches taries et les taures saillies (✓ **tout** ce qui s'applique):

(a) **Vaches en lactation:**	(b) **Vaches taries:**	(c) **Taures saillies**
☐ *Étable attachée*	☐ *Étable attachée*	☐ *Étable attachée*
☐ *Stabulation libre*	☐ *Stabulation libre*	☐ *Stabulation libre*
☐ *Litière accumulée*	☐ *Litière accumulée*	☐ *Litière accumulée*
☐ *Autre: (svp spécifier)*	☐ *Autre: (svp spécifier)*	☐ *Autre: (svp spécifier)*
.....	.....	.....

(d) Est-ce que les taures saillies et les vaches taries sont logées ensemble?
☐ *Oui* ☐ *Non*

(e) Si vous avez une étable à stabulation libre ou sur litière accumulée, combien d'espaces à la mangeoire y a-t-il (unités par vache)?

Vaches en lactation ..... *espaces*

Vaches taries ..... *espaces*

Taures saillies ..... *espaces*

(f) *Si vous avez une étable à stabulation libre,* combien de stalles les vaches ont-elles?

Vaches en lactation ..... *stalles*

Vaches taries ..... *stalles*

Taures saillies ..... *stalles*

1.3. (a) Est-ce que les **vaches en lactation** vont au pâturage durant l'été?
☐ *Non, elles restent dans l'étable à l'année longue*
☐ *Non, mais elles ont seulement accès à une cours d'exercice (moins de 5 acres / 100 vaches)*
☐ *Oui, elles vont au pâturage à partir du mois de ..... jusqu'au mois de.....*

(b) Durant la saison de pâturage, est-ce que vos vaches sont
☐ *À l'extérieur jour et nuit*
☐ *À l'intérieur la nuit seulement*
☐ *Autre: (svp spécifier)* .....

1.4. Avez-vous énoncé par écrit des objectifs de performance en santé du pis pour votre troupeau?
☐ *Oui* ☐ *Non*

1.4a. Est-ce que votre méthode de traite est consignée par écrit?
☐ *Oui* ☐ *Non*

1.5. (a) Quelle était votre moyenne de comptage des cellules somatiques (CCS) dans le lait en vrac l'an dernier?

..... *cellules/ml*

(b) À quel moment votre CCS est-il le plus élevé dans le lait en vrac? (✓ **toutes** les réponses qui s'appliquent)

- ☐ *Hiver*
- ☐ *Printemps*
- ☐ *Été*
- ☐ *Automne*
- ☐ *Pareil toute l'année*

---

2. Méthodes de traite et régie des cas de mammite

2.1. À quelle fréquence faites-vous la traite?

☐ *Deux fois par jour* ☐ *Trois fois par jour* ☐ *Autre: (svp spécifiez)* .....

2.2. À quelles heures effectuez-vous la traite approximativement?

1ère traite commence à (heure) _____ *et finit à* _____

2e traite commence à (heure) _____ *et finit à* _____

3e traite commence à (heure) _____ *et finit à* _____

2.3. Combien de personnes différentes ont trait les vaches au cours de la dernière semaine (inclure les trayeurs temporaires ou d'occasion)?

..... *trayeurs (femmes)*

..... *trayeurs (hommes)*

2.3a. Quand formez-vous vos employés de traite?

- ☐ *Jamais*
- ☐ *Toujours juste après les avoir engagé*
- ☐ *Lorsque le besoin survient*
- ☐ *Autre: (svp spécifiez)* .....

2.4. (a) Effectuez-vous la préparation du pis **d'une manière ou d'une autre** avant de poser l'unité de traite? ☐ *Oui* ☐ *Non (svp passer à la question 2.9)*

(b) Utilisez-vous de l'eau pour nettoyer les trayons?

☐ *Oui* ☐ *Non (svp passer à la question 2.6)*

(c) *Si vous utilisez de l'eau*, qu'utilisez-vous pour sécher les trayons?

- ☐ *Rien, je ne sèche pas les trayons*
- ☐ *Un linge ou une serviette*
- ☐ *Une serviette en papier ou du papier journal*
- ☐ *Autre: (svp spécifier)* .....

(d) Combien de vaches séchez-vous par serviette / linge?

..... *vaches*

2.5. (a) Utilisez-vous le **bain de trayon** ou la **pulvérisation avant la traite**?

☐ *Oui* ☐ *Non (svp passer à la question 2.7)*

(b) Quelle marque utilisez-vous?

☐ *Della PreTech® (DeLaval)*
☐ *Theratech® Pre & Post (WestfaliaSurge)*
☐ *Autre: (svp spécifiery)* .....

(c) De quelle façon essuyez-vous les trayons après le bain de trayon ou la pulvérisation?

☐ *Aucune, je n'essuie pas les trayons après le bain de trayon / pulvérisation*
☐ *Chiffon ou lavette*
☐ *Serviette de papier ou papier journal*
☐ *Autre: (svp spécifiery)* .....

(d) Combien de vaches sont-elles séchées avec la même serviette, lavette ou éponge?

..... *vaches*

2.6. (a) Si vous n'effectuez **pas** le bain de trayon ou la pulvérisation avant la traite, **essuyez-vous** les trayons?

☐ *Oui* ☐ *Non (svp passer à la question 2.8)*

(b) Qu'utilisez-vous pour essuyer les trayons?

☐ *Des serviettes humides disponibles commercialement, du genre ReadyWipe®*
☐ *Serviette ou linge sec*
☐ *Éponge*
☐ *Linges ou serviettes trempés dans l'eau (avec ou sans désinfectant)*
☐ *Autre: (svp spécifiery)* .....

(c) Combien de vaches sont-elles séchées avec la même serviette, lavette ou éponge?

..... *vaches*

2.7. (a) **Égouttez-vous** les trayons avant la traite?

☐ *Oui*
☐ *Non*
☐ *Seulement lorsque j'ai des problèmes de mammite*

(b) Si vous égouttez les trayons avant la traite, quand le faites-vous? (✓ **tout** ce qui s'applique)

☐ *À toutes les vaches, à chaque traite*
☐ *Seulement sur les vaches qu'on soupçonne d'avoir une mammite*
☐ *Sur les vaches avec un haut CCS*
☐ Sur les v*aches avec une mammite clinique*
☐ *Autre: (svp spécifiery)* .....

2.8. Effectuez-vous le **bain de trayon après la traite** (ou la pulvérisation)?

☐ *Oui* ☐ *Non (svp passer à la question 2.10)*

(b) Qu'utilisez-vous?

☐ *Le trempage*
☐ *La pulvérisation manuelle*
☐ *La pulvérisation automatique*
☐ *Autre: (svp spécifiery)* .....

(c) Quelle marque de bain de trayon utilisez-vous?

- ☐ *Della One® (DeLaval)*
- ☐ *Della Soft® (DeLaval)*
- ☐ *Teat-Kote® (WestfaliaSurge)*
- ☐ *Bovi-Kote® (Bou-Matic)*
- ☐ *Emerald® (ABS Global)*
- ☐ *Protek® (Ecolab)*
- ☐ *Mastimin 50 Dripless®*
- ☐ *Uddergold® (Ecolab)*
- ☐ *Theratec Pre & Post® (WestfaliaSurge)*
- ☐ *Autre: (svp spécifiery)* .....

(d) Effectuez-vous le bain de trayon (ou pulvérisation) après la traite à l'année longue?

☐ *Oui*

☐ *Non, je n'effectue pas le bain de trayon ou la pulvérisation après la traite du ..... à .....*

2.9. Votre équipement de traite est-il muni du système de retrait automatique?

(a) ☐ *Oui (svp passer à la question 2.9c)* ☐ *Non*

(b) Coupez-vous le vide avant de détacher les manchons-trayeurs?

☐ *Oui* ☐ *Non*

(c) À quel débit votre système est-il ajusté pour le retrait des unités?

☐ *Je ne sais pas*

☐ *À ..... kg / min*

2.10. Est-ce que vos trayeurs et vous-même portez des gants de latex (ou similaire) durant la traite?

☐ *Oui* ☐ *Parfois* ☐ *Non*

2.11. Pour combien de vaches devez-vous exercer des mesures de contention pour la traite?

..... *vaches*

2.12. (a1) Trayez-vous les vaches ayant un comptage de cellules somatiques (CCS) élevé en dernier et/ou avec une unité séparée?

☐ *Oui* ☐ *Non*

(a2) Nettoyez-vous l'unité de traite après avoir trait une vache avec un comptage de cellules somatiques élevé?

☐ *Oui, après chaque vache*

☐ *Parfois*

☐ *Non*

(b1) Trayez-vous les vaches infectées à Staphylococcus aureus en dernier et/ou avec une unité séparée?

☐ *Oui* ☐ *Non*

(b2) Nettoyez-vous l'unité de traite après avoir trait une vache infectée à *Staphylococcus aureus?*

☐ *Oui, après chaque vache*

☐ *Parfois*

☐ *Non*

(c1) Trayez-vous les vaches ayant une mammite clinique en dernier **et / ou** avec une unité séparée?

☐ *Oui* ☐ *Non*

(c2) Nettoyez-vous l'unité de traite après avoir trait une vache **ayant une mammite clinique**?

☐ *Oui, après chaque vache*

☐ *Parfois*

☐ *Non*

2.13. Quel moyen utilisez-vous pour empêcher les vaches de se coucher après la traite?

☐ *Aucun*
☐ *Je distribue des aliments frais*
☐ *Je verrouille les portes cornadis*
☐ *Je laisse les vaches debout dans une aire d'attente*
☐ *Autre: (svp spécifiez) .....*

2.14. Après combien de jours suivant le vêlage mettez-vous le lait de la vache fraîche dans le réservoir?

..... jours

---

3. Régie des cas de mammite ciinique

3.1. Selon vous, combien de cas de mammite avez-vous par mois?

Environ ..... cas / mois

3.2. Attribuez-vous la présence de sang dans le lait à la mammite?

☐ *Oui* ☐ *Non*

3.3. Attribuez-vous un lait anormal juste après le vêlage à la mammite?

☐ *Oui* ☐ *Non*

3.4. Êtes-vous en accord avec les énoncés suivants?

	En désaccord	*Partiellement en désaccord*	*Neutre*	*Partiellement en accord*	*D'accord*
(a) Quand elle survient, la mammite clinique est facile à dépister	1☐	2☐	3☐	4☐	5☐
(b)Je suis préoccupé par les coûts de la mammite clinique	1☐	2☐	3☐	4☐	5☐
(c) Les cultures des échantillons de lait dans les cas de mammite clinique sont inutiles	1☐	2☐	3☐	4☐	5☐
(d) Je m'assure toujours de terminer le traitement (tel que recommandé)	1☐	2☐	3☐	4☐	5☐
(e) De nos jours, je crois que les antibiotiques ne sont pas aussi efficaces qu'avant	1☐	2☐	3☐	4☐	5☐
(f) Il est souvent nécessaire de changer d'antibiotique en cours de traitement	1☐	2☐	3☐	4☐	5☐

3.5. De quelle(s) façon(s) les cas de mammite clinique sont-ils communément observés ou détectés sur votre ferme?

	Rarement		*Neutre*		*Très souvent*
(a) Lait anormal	1☐	2☐	3☐	4☐	5☐
(b) Pis anormal	1☐	2☐	3☐	4☐	5☐
(c) Agitation anormale durant la traite (coups de patte)	1☐	2☐	3☐	4☐	5☐
(d) Vache malade	1☐	2☐	3☐	4☐	5☐
(e) En utilisant un conductimètre (automatisé)	1☐	2☐	3☐	4☐	5☐
(f) Autre: (svp spécifiez)	1☐	2☐	3☐	4☐	5☐

3.6. Traitez-vous tous les cas de mammite avec des antibiotiques?

☐ *Dans tous les cas*
☐ *Quelques cas, environ ..... % (svp spécifier)*
☐ *En aucun cas*

3.7 Désinfectez-vous le trayon avec un tampon d'alcool avant l'infusion?

☐ *Oui*
☐ *Parfois*
☐ *Non*

3.8. Utilisez-vous l'insertion complète (long bout) ou partielle (court bout)?

☐ *Complète*
☐ *Partielle*

3.9. Quel est le nombre **minimum** de traitements antibiotiques que vous administrer à une vache souffrant de mammite?

Minimum de ..... traitements

3.10. Quel est le nombre **maximum** de traitements que vous administrez à une vache si les symptômes persistent?

Maximum de ..... traitements

3.11. À quelle fréquence trayez-vous une vache souffrant de mammite clinique?

☐ *Seulement durant la traite habituelle*
☐ *3 – 4 fois par jour*
☐ *plus de 4 fois par jour*
☐ *Autre: (svp spécifiez) .....*

3.12. Dans cette étude, pourriez-vous nous donner une idée du nombre de cas de mammite clinique que vous pourriez avoir oublié ou qui vous auraient échappés pour prendre un échantillon?

*Je n'ai **pas** échantillonné environ ..... cas (svp spécifier)*

3.13. Selon vous, quel est le coût moyen d'un cas de mammite clinique?

Un cas de mammite clinique coûte environ .....$ (svp spécifiez)

3.14. De quelle façon vous souvenez-vous ou notez-vous qu'une vache a reçu un traitement? (√ **tout** ce qui s'applique)

☐ *Le nom ou le numéro de la vache sur un tableau blanc ou noir à la craie?*
☐ *Garder la vache séparée*
☐ *Apposer un bracelet coloré sur une patte*
☐ *Marquer la vache d'une couleur (jambe, dos, pis, queue, etc.)*
☐ *Autre: (svp spécifiez) .....*

3.15. (a) Vaccinez-vous vos vaches contre la mammite?

☐ Toutes les vaches
☐ La plupart des vaches (≥ 50%)
☐ Quelques vaches (< 50%)
☐ *Aucune (svp passer à la question 3.16)*

(b) Quand vaccinez-vous?

☐ *Au tarissement*
☐ *Avant le vêlage*
☐ *En début de lactation (0-100 JEL)*
☐ *En milieu de lactation (101-200 JEL)*
☐ *Autre: (svp spécifiez) .....*

3.16. Combien de vos vaches ont été réformées à cause de la mammite dans la dernière année?

..... *vaches*

3.17. En collaboration avec leur médecin vétérinaire, certains producteurs ont élaboré *un plan thérapeutique spécifique* basé sur la sensibilité des bactéries présentes sur leur ferme et sur leurs problèmes spécifiques.

(a) Avez vous *un plan thérapeutique spécifique* à votre ferme écrit sur papier ou à l'ordinateur?
☐ *Oui* ☐ *Non*

(b) Croyez-vous qu'*un plan thérapeutique spécifique* à votre ferme pourrait être utile?
☐ *Oui* ☐ *Peut-être* ☐ *Non*

(c) Seriez-vous intéressé à élaborer votre propre *plan thérapeutique spécifique* en collaboration avec votre médecin vétérinaire?

Non	*Probablement pas*	*Neutre*	*Peut-être*	*Oui*
1☐	2☐	3☐	4☐	5☐

4. Régie des vaches taries

4.1. (a) Quelle proportion de vos vaches reçoivent un traitement au tarissement avec des antibiotiques à la fin de la lactation?

Environ ..... %

(b) Quels produits utilisez-vous en période de tarissement? (✓ **tout** ce qui s'applique)
☐ *Dryclox®*
☐ *Cefadry®*
☐ *Novodry®*
☐ *Autre: (svp spécifiez)* .....

4.2. Désinfectez-vous le trayon avec un tampon d'alcool avant l'infusion?
☐ *Oui*
☐ *Parfois*
☐ *Non*

4.3. Utilisez-vous l'insertion complète (long bout) ou partielle (court bout)?
☐ *Complète*
☐ *Partielle*

4.4. (a) Utilisez-vous *Orbeseal®* au tarissement?
☐ *Oui* ☐ *Non*

(b) Quelle proportion des vaches sont traitées avec *Orbeseal®* au tarissement?

Environ. ..... %

(c) Utilisez-vous *Orbeseal®* en combinaison avec des antibiotiques?

☐ *Oui, toujours en combinaison avec des antibiotiques*
☐ *Parfois*
☐ *Non, j'utilise toujour Orbeseal® seul*

4.5. Diminuez-vous la fréquence de traite durant la semaine précédant le tarissement?
☐ *Oui* ☐ *Non*

4.6. Observez-vous régulièrement les **vaches taries** pour détecter les signes visibles de mammite clinique?
☐ *Non, jamais*
☐ *Oui, à tous les ..... jours (svp spécifiez la fréquence)*

4.7. Observez-vous régulièrement les taures saillies pour détecter les signes visibles de mammite clinique?
☐ *Non, jamais*
☐ *Oui, à tous les ..... jours (svp spécifiez la fréquence)*

4.8. Quelle est la durée moyenne des période de tarissement?

..... *jours*

---

5. Vaches avec un comptage de cellules somatiques élevé

5.1. À quel niveau du comptage de cellules somatiques considérez-vous qu'une vache a un comptage **élevé**?

.....*cellules/ml et plus*

5.2. Êtes-vous en accord avec les énoncées suivants?

	En désaccord		*Neutre*		*En accor*
(a) Les vaches avec un CCS élevé sont faciles à identifier durant la traite	1☐	2☐	3☐	4☐	5☐
(b) Je suis préoccupé par les coûts associés aux vaches avec un CCS élevé	1☐	2☐	3☐	4☐	5☐
(c) La culture des échantillons de lait des vaches avec un CCS élevé est généralement inutile	1☐	2☐	3☐	4☐	5☐

5.3. Les méthodes les plus importantes pour détecter les vaches avec un CCS élevé sont:: (✓ **toutes** les réponses qui s'appliquent)

☐ *CCS individuel sur le rapport de contrôle laitier*
☐ *Observation de la vache et de son pis*
☐ *Évaluation automatisée (conductivité)*
☐ *Utilisation du CMT (Test de Californie)*
☐ *Autre: (svp spécifiez)* .....

5.4. Combien de vaches avez-vous réformé à cause d'un CCS élevé dans la dernière année?

..... *vaches*

5.5. Récoltez-vous des échantillons de lait des vaches avec un **CCS élevé** pour les cultures bactériennes?
☐ *Toutes les vaches*
☐ *La plupart (≥ 50%)*
☐ *Quelques-unes (<50%)*
☐ *Aucune*

---

6. Équipement de traite

6.1. Le niveau de vide durant la traite est:

☐ ..... *kPa ou .....mm Hg ou .....inch Hg ou ..... psi*
☐ *Je ne sais pas exactement*

6.2. Si vous avez une étable à **stalles entravées,** quel type de système de traite possédez-vous?
☐ *Lactoduc*
☐ *Chaudières*

6.3. *Si vous* **n'avez pas** *une étable à stalles entravées,*
(a) Quel type de salle de traite avez-vous?
☐ *Salle de traite en épis*
☐ *Salle de traite parallèle*
☐ *Salle de traite avec stalle individuelle*
☐ *Système de traite automatisé (Robot)*
☐ *Rotatif*
☐ *Autre: (svp spécifiez)* .....

(b) Quel type de lactoduc avez-vous?
☐ *Ligne haute*
☐ *Ligne basse*
☐ *Autre: (svp spécifiez)* .....

(c) Les vaches ont-elles accès à de l'eau dans l'aire d'attente avant la traite?
☐ *Oui* ☐ *Non*

6.4. Combien avez-vous d'unités dans votre système de traite (étable entravée ou salon de traite)?

..... *unités de traite*

6.5. À quelle fréquence faites-vous vérifier et analyser le fonctionnement de votre équipement de traite par le marchand d'équipements?
☐ *Deux fois ou plus par année*
☐ *Une fois par année*
☐ *Moins d'une fois par année*
☐ *Jamais*

6.6. À quelle fréquence faites-vous vérifier et analyser le fonctionnement de votre équipement de traite par *un technicien indépendant?*
☐ *Deux fois ou plus par année*
☐ *Une fois par année*
☐ *Moins d'une fois par année*
☐ *Jamais*

6.7. À quelle fréquence vérifiez-vous le niveau de vide?
☐ *Jamais*
☐ *Seulement si j'ai des problèmes de mammite*
☐ *Une fois par mois*
☐ *Une fois par semaine*
☐ *Presque chaque jour*
☐ *Autre: (svp spécifiez)* .....

6.8. Avez-vous fait inspecter votre étable / ou votre équipement de traite pour les tensions parasites au cours des 2 dernières années?

☐ *Oui* ☐ *Non*

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7. Confort et hygiène de la vache

7.1. Quel matériel est utilisé comme base de la stalle (✓ **tout** ce qui s'applique)?

(a) **Vaches en lactation:**	(b) **Vaches taries:**	(c) **Taures saillies:**
☐ *Ciment*	☐ *Ciment*	☐ *Ciment*
☐ *Matelas*	☐ *Matelas*	☐ *Matelas*
☐ *Matelas en caoutchouc*	☐ *Matelas en caoutchouc*	☐ *Matelas en caoutchouc*
☐ *Argile*	☐ *Argile*	☐ *Argile*
☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*
.....	.....	.....

7.2. Quel type de litière utilisez-vous (✓ **tout** ce qui s'applique)?

(a) **Vaches en lactation:**	(b) **Vaches taries:**	(c) **Taures saillies:**
☐ *Aucune*	☐ *Aucune*	☐ *Aucune*
☐ *Sciure de bois*	☐ *Sciure de bois*	☐ *Sciure de bois*
☐ *Copeaux de bois*	☐ *Copeaux de bois*	☐ *Copeaux de bois*
☐ *Sable*	☐ *Sable*	☐ *Sable*
☐ *Paille*	☐ *Paille*	☐ *Paille*
☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*
.....	.....	.....

7.3. À quelle fréquence **nettoyez-vous le fumier** dans les stalles? (par exemple gratter la moitié arrière hors des stalles) (✓ **tout** ce qui s'applique)

(a) **Vaches en lactation:**	(b) **Vaches taries:**	(c) **Taures saillies:**
☐ *Deux fois par jour*	☐ *Deux fois par jour*	☐ *Deux fois par jour*
☐ *Une fois par jour*	☐ *Une fois par jour*	☐ *Une fois par jour*
☐ *Une fois par deux jours*	☐ *Une fois par deux jours*	☐ *Une fois par deux jours*
☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*
.....	.....	.....

7.4. À quelle fréquence nettoyez-vous le fumier dans les stalles (✓ **tout** ce qui s'applique)?

(a) **Vaches en lactation:**	(b) **Vaches taries:**	(c) **Taures saillies:**
☐ *Une fois par jour*	☐ *Une fois par jour*	☐ *Une fois par jour*
☐ *Une fois par deux jours*	☐ *Une fois par deux jours*	☐ *Une fois par deux jours*
☐ *Deux fois par semaine*	☐ *Deux fois par semaine*	☐ *Deux fois par semaine*
☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*
.....	.....	.....

7.5. *Si vous avez une stabulation libre,* de quelle(s) façon(s) nettoyez-vous les allées?

(✓ **tout** ce qui s'applique)

(a) ☐ *Nettoyage manuel*
☐ *Raclette automatique*
☐ *Chargeur frontal ou tracteur*
☐ *Autre: (svp spécifiez) .....*

(b) À quelle fréquence les allées sont-elles nettoyées (manuel ou raclette)

..... fois par jour

7.6. Coupez-vous le poil du pis (à la torche ou avec une tondeuse) et à quelle fréquence le faites-vous?
☐ *Non*
☐ *Tondeuse, ... fois / années*
☐ *Torche, ... fois / années*

7.7. Rasez-vous ou coupez vous les queues?
☐ *Non*
☐ *Raser ..... fois / années*
☐ *Couper*

7.8. (a) Avez-vous un **parc de vêlage**?
☐ *Oui* ☐ *Non (svp passez à la question 8.1)*

(b) Utilisez-vous le même parc pour les vaches malades?
☐ *Oui* ☐ *Non*

(c) Quel type de litière utilisez-vous dans le parc de vêlage? (✓ **toutes** les réponses qui s'appliquent)
☐ *Aucune*
☐ *Sciures de bois*
☐ *Copeaux de bois*
☐ *Sable*
☐ *Paille*
☐ *Autre: (svp spécifiez) .....*

(d) À quelle fréquence remplacez-vous la litière du parc par de la litière propre?
☐ *Après chaque vêlage*
☐ *Autre: (svp spécifiez) .....*

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8. Biosécurité et prévention

8.1. Est-ce que les visiteurs doivent désinfecter leurs bottes ou leurs souliers avant d'entrer dans votre étable?
☐ *Oui* ☐ *Non*

8.2. Est-ce que les visiteurs de votre étable doivent revêtir les vêtements protecteurs que vous leur fournissez (ex. bottes, combinaison)?
☐ *Oui* ☐ *Non*

8.3. (a) Combien de vos taures et de vos vaches ont-elles été achetées?

... ... taures achetées ..... *vaches achetées*

(b) Si vous achetez des vaches, récoltez-vous et analysez-vous des échantillons de leur lait avant l'achat?

- ☐ *Je prends toujours des échantillons de lait*
- ☐ *Habituellement (≥50%)*
- ☐ *Parfois (<50%)*
- ☐ *Jamais*

8.4. (a) Administrez-vous un traitement antibiotique à vos taures comme mesure de prévention avant le vêlage?

- ☐ *Oui*
- ☐ *Parfois*
- ☐ *Non (svp passer à la question 8.5)*

(b) De quelle façon le faites-vous?

- ☐ *Dans le muscle (dans le cou, à la croupe, etc.)*
- ☐ *Dans le pis avec un traitement pour les vaches taries*
- ☐ *Dans le pis avec un traitement pour les vaches en lactation*
- ☐ *Autre: (svp spécifier)* .....

8.5. Êtes-vous en accord avec les énoncés suivants?

	En désaccord			*Neutre*				*En accor*
(a) Je surveille toujours de très près le CCS du lait en vrac	1☐	2☐	3☐	4☐	5☐
(b) Si je le veux, je peux baisser le CCS de mon lait en vrac	1☐	2☐	3☐	4☐	5☐
(c) Je crois qu'une analyse du CCS individuel de chaque vache est très importante	1☐	2☐	3☐	4☐	5☐
(d) J'aimerais diminuer le nombre de vaches qui ont une mammite	1☐	2☐	3☐	4☐	5☐
(e)Je connais généralement les causes d'augmentation des cas de mammite dans ma ferme	1☐	2☐	3☐	4☐	5☐
(f) Généralement, on ne peut influencer les causes de la mammite	1☐	2☐	3☐	4☐	5☐
(g) La malchance est un facteur important dans les épisodes de mammite	1☐	2☐	3☐	4☐	5☐

8.6. Lors d'un épisode de mammite à ***Staphylococcus aureus,*** il est important de:

- ☐ *Garder les stalles très propres car cette bactérie se propage principalement dans le fumier, la litière et l'environnement de la vache.*
- ☐ *Porter une attention particulière à l'hygiène durant toutes les étapes de la traite.*
- ☐ *Je ne sais pas*

8.7. Lors d'un épisode de mammite à ***E. coli***, il est important de:

- ☐ *Garder les stalles très propres car cette bactérie se propage principalement dans le fumier, la litière et l'environnement de la vache.*
- ☐ *Porter une attention particulière à l'hygiène durant toutes les étapes de la traite.*
- ☐ *Je ne sais pas*

8.8. Êtes-vous en accord avec les énoncés suivants:

	En désaccord		Neutre		En accord
(a) *Si je constate une augmentation soudaine des cas de mammite, je veux savoir quelles bactéries sont en cause*	1☐	2☐	3☐	4☐	5☐
(b) Je crois que les analyses bactériologiques sont trop coûteuses	1☐	2☐	3☐	4☐	5☐
(c) Je trouve que le délai est trop long avant de recevoir les résultats d'analyse des échantillons soumis au laboratoire	1☐	2☐	3☐	4☐	5☐
(d) L'interprétation des résultats de laboratoire est difficile	1☐	2☐	3☐	4☐	5☐
(e) Les analyses bactériologiques sont importantes car elles déterminent l'orientation à prendre pour le traitement	1☐	2☐	3☐	4☐	5☐
(f) Le traitement et la prévention des mammites sont importants dans ma ferme	1☐	2☐	3☐	4☐	5☐
(g) J'ai suffisamment de connaissances sur la mammite pour éviter d'avoir des problèmes	1☐	2☐	3☐	4☐	5☐
(h) Je devrais me consacrer davantage à la prévention de la mammite	1☐	2☐	3☐	4☐	5☐
(I) Je n'ai pas assez de temps pour faire de la prévention de mammite	1☐	2☐	3☐	4☐	5☐

8.9. Utilisez-vous un ordinateur pour la tenue de dossiers de vos vaches?

☐ *Oui* ☐ *Non*

8.10. Quel système utilisez-vous pour la tenue de dossier des *cas de mammite clinique?*

(✓ **tout** ce qui s'applique)

☐ *Aucun*
☐ *Tableau blanc ou noir à craie ou similaire*
☐ *Cartes de vaches*
☐ *Tableau de régie*
☐ *Calendrier de 21 jours*
☐ *Carnet de régie*
☐ *Ordinateur*
☐ *Autre: (svp spécifiez)* .....

8.11. Quelles données consignez-vous dans les cas de mammite clinique? (✓ **tout** ce qui s'applique)

Nom ou numéro de la vache	☐ *Oui*	☐ *Non*
Quel quartier est affecté	☐ *Oui*	☐ *Non*
Sévérité	☐ *Oui*	☐ *Non*
Date de début des signes	☐ *Oui*	☐ *Non*
Date du dernier traitement	☐ *Oui*	☐ *Non*
Type de traitement	☐ *Oui*	☐ *Non*
Nombre de traitements	☐ *Oui*	☐ *Non*
Date de retour dans le réservoir de lait	☐ *Oui*	☐ *Non*
Types de bactéries observées à la culture	☐ *Oui*	☐ *Non*

9. Nutrition

9.1. Est-ce que la ration distribuée aux vache est une RTM (ration totale mixte)?
☐ *Oui* ☐ *Non*

9.2. À quelle fréquence balancez-vous les rations des vaches en vous basant sur les analyses de fourrages?
☐ *Trois fois ou plus par année*
☐ *Deux fois par année*
☐ *Une fois par année*
☐ *Moins d'une fois par année*
☐ *Jamais*

9.3. Alimentez-vous vos vaches en lactation avec:

Ensilage de maïs	☐ *Oui*	☐ *Non*
Pommes de terre	☐ *Oui*	☐ *Non*
Pulpe de betterave	☐ *Oui*	☐ *Non*

9.4. Alimentez-vous vos vaches taries avec les restes de vos vaches en lactation aux vaches en lactation?
☐ *Oui* ☐ *Non*

9.5. À quel stade de la lactation vos vaches sont-elles nourries avec les niveaux d'énergie les plus élevés?

☐ *Environ de ..... JEL à ..... JEL*

9.6. À combien de jours avant le tarissement réduisez-vous les **niveaux d'énergie alimentaire**?
☐ *Pas de réduction d'énergie ou d'aliments*

☐ ..... *jours avant le tarissement (svp donner le nombre de jours)*

9.7. À combien de jours avant le tarissement réduisez-vous l'**apport d'eau**?
☐ *Pas de réduction de l'apport d'eau*

☐ ..... *jours avant le tarissement (svp donner le nombre de jours)*

9.8. (a) Utilisez-vous des additifs de minéraux et d'oligo-éléments *dans la ration*?
☐ *Oui* ☐ *Non (svp passez à la question 9.9)*

(b) Quels additifs utilisez-vous?

(I) **Vaches en lactation:**
☐ *Mélange commercial*
☐ *Préparations de multivitamines*
☐ *Vitamine E*
☐ *Sélénium (Se)*
☐ *Cuivre (Cu)*
☐ *Magnésium (Mg)*
☐ *Sodium (Na)*
☐ *Potassium (K)*
☐ *Calcium (Ca)*

(II) **Vaches taries:**
☐ *Mélange commercial*
☐ *Préparations de multivitamines*
☐ *Vitamine E*
☐ *Sélénium (Se)*
☐ *Cuivre (Cu)*
☐ *Magnésium (Mg)*
☐ *Sodium (Na)*
☐ *Potassium (K)*
☐ *Calcium (Ca)*

(III) **Taures saillies:**
☐ *Mélange commercial*
☐ *Préparations de multivitamines*
☐ *Vitamine E*
☐ *Sélénium (Se)*
☐ *Cuivre (Cu)*
☐ *Magnésium (Mg)*
☐ *Sodium (Na)*
☐ *Potassium (K)*
☐ *Calcium (Ca)*

(I) **Vaches en lactation:**	(II) **Vaches taries:**	(III) **Taures saillies:**
☐ *Rumensin®*	☐ *Rumensin®*	☐ *Rumensin®*
☐ *Niacin®*	☐ *Niacin®*	☐ *Niacin®*
☐ *Levure*	☐ *Levure*	☐ *Levure*
☐ *Varech ou algues*	☐ *Varech ou algues*	☐ *Varech ou algues*
☐ *Zinpro®*	☐ *Zinpro®*	☐ *Zinpro®*
☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*	☐ *Autre: (svp spécifiez)*
.....	.....	.....

9.9. (a) Donnez-vous des injections de minéraux / vitamines / oligo-éléments à vos vaches?
☐ *Oui* ☐ *Non (svp passez à la question 9.10)*

(b) Quels minéraux / vitamines / oligo-éléments injectez-vous à vos vaches?
☐ *Vitamine B (n'importe quelle)*
☐ *Vitamine D (n'importe quelle)*
☐ *Vitamine E / Sélénium*
☐ *Préparations de multivitamines*
☐ *Autre: (svp spécifiez)* .....

9.10. Pouvez-vous indiquer le rôle des intervenants suivants dans la formulation des rations de vos vaches:

	Pas important			*Neutre*		*Très important*
(a) Nutritionniste indépendant	1☐	2☐	3☐	4☐	5☐
(b) Représentant de compagnie d'alimentation	1☐	2☐	3☐	4☐	5☐
(c) *Médecin vétérinaire*	1☐	2☐	3☐	4☐	5☐
(d) *Représentant d'ATLC / DHI (PATLQ, Canwest DHI, ou ADLIC)*	1☐	2☐	3☐	4☐	5☐
(e) *Autre: (svp spécifiez)*	1☐	2☐	3☐	4☐	5☐

9.11. Quelle est la source d'eau des vaches?
☐ *Puit creusé*
☐ *Puit foré*
☐ *Eau de surface (ruisseau, rivière, lac, étang, etc.)*
☐ *Eau de ville*
☐ *Autre: (svp spécifiez)* .....

9.12. Avez-vous effectué une analyse d'eau au cours des 2 dernières années?
(a) Analyse des bactéries ..... ☐ *Oui* ☐ *Non*
(b) Analyse des minéraux ..... ☐ *Oui* ☐ *Non*

(c) Si vous avez avez effectué une analyse des bactéries, y avait-il des problèmes avec la qualité de votre eau?
☐ *Oui* ☐ *Non*

10. Révision et communication du plan de régie de la mammite

10.1. Qui est la personne importante pour réviser vos données et votre plan de régie de la mammite avec vous?

	Pas important		*Neutre*		*Très important*
(a) Médecin vétérinaire	1☐	2☐	3☐	4☐	5☐
(b) Représentant d'ATLC / DHI (PATLQ, Canwest DHI, ou ADLIC)	1☐	2☐	3☐	4☐	5☐
(c) Nutritionniste	1☐	2☐	3☐	4☐	5☐
(d) Représentant d'équipement de traite	1☐	2☐	3☐	4☐	5☐
(e) Autres producteurs	1☐	2☐	3☐	4☐	5☐
(f) Membre (s) de la famille	1☐	2☐	3☐	4☐	5☐
(g) Autre: (svp spécifiez)	1☐	2☐	3☐	4☐	5☐

10.2. (a) Révisez vous votre rapport de contrôle laitier le jour même de la réception?

☐ *Oui* ☐ *Non*

(b) À quelle fréquence prenez-vous le temps de vous asseoir pour réviser vos données de mammite? (✓ **toutes** les réponses qui s'appliquent)

☐ *Une fois par semaine*
☐ *Deux fois par mois*
☐ *Une fois par mois*
☐ *Deux fois par année*
☐ *Seulement lorsque le CCS du lait en vrac dépasse..... cellules/mL (svp spécifiez)*
☐ *Autre: (svp spécifiez) .....*

10.3. Si votre médecin vétérinaire organisait un cours sur la prévention de la mammite s'étalant sur 2 jours, seriez-vous intéressé à participer (moyennant des frais d'inscription)?

☐ *Non*
☐ *Oui et je payerais un maximum de ....$*

10.4. Combien de temps consacrez-vous par semaine à la lecture de littérature sur la régie de la santé des vaches?

Environ. ..... heures

10.5. Quelle publication préférez-vous lire? (✓ **toutes** les réponses qui s'appliquent)

☐ *Hoard's Dairyman*
☐ *The Milk Producer*
☐ *Le producteur de lait québécois*
☐ *Western Dairy Digest*
☐ *Autre: (svp spécifiez) .....*

10.6. Êtes-vous en accord avec les énoncés suivants?

	En désaccord		*Neutre*		*En accord*
(a) Je serais certainement intéressé à lire des articles sur la mammite dans mes magazines de production laitière.....	1☐	2☐	3☐	4☐	5☐
(b) J'aimerais qu'il y ait davantage d'articles sur la régie de troupeau dans mes magazines de production laitière	1☐	2☐	3☐	4☐	5☐

10.7. Si vous vouliez en apprendre davantage sur la prévention de la mammite, quelles seraient les **trois (3)** façons les plus appropriées pour vous:

☐ *Articles dans les magazines de production laitière*
☐ *Site Internet spécial sur la mammite*
☐ *Discussions avec d'autres producteurs laitiers*
☐ *Un symposium avec un expert sur la mammite*
☐ *Conseil de mon médecin vétérinaire*
☐ *Lire un livre ou un guide sur la mammite*
☐ *Service d'assistance gratuit*
☐ *Cours sur vidéo*
☐ *Programme d'auto-assistance sur CD-rom*
☐ *Une visite régulière d'un expert de la mammite à ma ferme*
☐ *Un cours sur la mammite*

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11. Questions générales finales

11.1. À partir de quel niveau du **comptage de cellules somatiques du lait en vrac** croyez-vous avoir un problème de mammite ?

À .....,000 cellules/mL

11.2. À quel niveau d'incidence de la **mammite clinique** croyez-vous avoir un problème de mammite (ici la "mammite clinique" se définit par une "anormalité visible dans le lait et/ou le pis")?

*Lorsque le nombre de mammites cliniques dépasse ..... cas par **mois***

11.3. À quel niveau d'incidence des vaches avec un nouveau **comptage de cellules somatiques élevé** (toutes les vaches avec un CCS plus haut que 200,000 cellules/mL) croyez-vous avoir un problème de mammite?

Lorsque le nombre de vaches avec un nouveau comptage de cellules somatiques élevé dépasse

..... *cas par **mois***

11.4. Avez-vous déjà eu un problème de mammite tel que décrit ci-haut?

☐ *Oui* ☐ *Non*

11.5. Avez-vous eu des problèmes de mammite dans les 2 dernières années?

Peu		*Moyen*		*Beaucoup*
1☐	2☐	3☐	4☐	5☐

11.6. Êtes-vous en accord avec les énoncés suivants?

	En désaccord		*Neutre*		*En accord*
(a) En général, je gère bien la mammite dans ma ferme	1☐	2☐	3☐	4☐	5☐
(b) La mammite est une maladie difficile à gérer pour moi	1☐	2☐	3☐	4☐	5☐
(c) Tous les cas de mammite m'inquiètent beaucoup ...	1☐	2☐	3☐	4☐	5☐
(d) Tous les cas de mammite engendrent beaucoup de travail supplémentaire	1☐	2☐	3☐	4☐	5☐

11.7. Quel est l'aspect le plus dérangeant de la mammite pour vous? (**une** seule réponse svp)

- ☐ *Dérangement dans ma routine de traite*
- ☐ *Conséquences financières*
- ☐ *Surplus de travail*
- ☐ Autre: *(svp spécifiez)* .....

11.8. Êtes-vous en accord avec les énoncés suivants?

	En désaccord		*Neutre*		*En accord*
(a) Je suis assez souvent préoccupé par la mammite	1☐	2☐	3☐	4☐	5☐
(b) Je crois que je gère la prévention et le traitement de la bonne façon	1☐	2☐	3☐	4☐	5☐
(c) Tant que les problèmes de mammite ne deviennent pas trop sérieux, je ne changerai rien	1☐	2☐	3☐	4☐	5☐
(d) J'ai changé mes habitudes de régie au cours des 5 dernières années à cause de problèmes de mammite	1☐	2☐	3☐	4☐	5☐
(e) La santé du pis est un caractère important dans la sélection des taureaux	1☐	2☐	3☐	4☐	5☐

11.9. Comment qualifieriez-vous votre niveau de connaissances sur les sujets suivants:

	Insuffisantes	*Moins que suffisantes*	*Suffisantes*	*Bonnes*	*Excellentes*
(a) Influence de la nutrition sur la mammite	1☐	2☐	3☐	4☐	5☐
(b) Équipement de traite	1☐	2☐	3☐	4☐	5☐
(c) Méthodes de traite	1☐	2☐	3☐	4☐	5☐
(d) Type et entretien des bâtiments	1☐	2☐	3☐	4☐	5☐
(e)Bonne utilisation des rapports de contrôle laitier pour la régie de la mammite	1☐	2☐	3☐	4☐	5☐
(f) Type de bactéries et cultures bactériennes	1☐	2☐	3☐	4☐	5☐
(g)Traitement de la mammite clinique	1☐	2☐	3☐	4☐	5☐
(h)Traitement de la mammite subclinique	1☐	2☐	3☐	4☐	5☐
(i) Utilisation des médicaments contre la mammite	1☐	2☐	3☐	4☐	5☐
(j) Politiques d'achat et de réforme des animaux	1☐	2☐	3☐	4☐	5☐

12.1. Quel est votre niveau d'intérêt pour les aspects suivants de l'élevage de vaches laitières:

	Aucun intérêt		*Neutre*		*Beaucoup d'intérêt*
(a) Régie des pâturages	1☐	2☐	3☐	4☐	5☐
(b) Amélioration génétique	1☐	2☐	3☐	4☐	5☐
(c) Économie et gestion des finances	1☐	2☐	3☐	4☐	5☐
(d) Gestion des minéraux et des oligo-éléments	1☐	2☐	3☐	4☐	5☐
(e) Nutrition	1☐	2☐	3☐	4☐	5☐
(f) Machinerie	1☐	2☐	3☐	4☐	5☐
(g) Santé animale	1☐	2☐	3☐	4☐	5☐
(h) Veaux/élevage de la relève	1☐	2☐	3☐	4☐	5☐
(i) Traite	1☐	2☐	3☐	4☐	5☐
(j) Planification du travail	1☐	2☐	3☐	4☐	5☐
(k) Autres revenus que la production laitière	1☐	2☐	3☐	4☐	5☐

12.2. Quelle importance ont ces buts dans votre ferme?

	Pas important			*Neutre*		*Très important*
(a) Haute production de lait par vache	1☐	2☐	3☐	4☐	5☐	
(b) Le plus grand nombre possible de vaches par acre	1☐	2☐	3☐	4☐	5☐	
(c) Le plus grand nombre possible de vaches par personne	1☐	2☐	3☐	4☐	5☐	
(d) Expansion de la ferme avec plus de terres	1☐	2☐	3☐	4☐	5☐	
(e) Expansion de la ferme avec plus de quota	1☐	2☐	3☐	4☐	5☐	
(f) Maintenir une gestion simple	1☐	2☐	3☐	4☐	5☐	
(g) Avoir le moins de dettes possible	1☐	2☐	3☐	4☐	5☐	
(h) Avoir les rendements les plus hauts possible	1☐	2☐	3☐	4☐	5☐	
(i) Avoir un revenu autre que la production laitière	1☐	2☐	3☐	4☐	5☐	
(j) Planifier une succession facile à mon fils ou ma fille	1☐	2☐	3☐	4☐	5☐	

12.3. (a) Croyez-vous qu'une prime devrait être payée pour les comptages de cellules somatiques bas dans le lait ?

☐ *Oui* ☐ *Non* ☐ *Pas d'opinion sur le sujet*

(b) Changeriez-vous vos habitudes de régie dans le but de recevoir cette prime?

☐ *Oui* ☐ *Peut-être* ☐ *Non*

12.4. Avez-vous d'autres commentaires? (*Svp utilisez l'envers de la page pour vos commentaires additionnels)*

Merci pour votre collaboration!

APPENDIX 3

Canadian Bovine Mastitis
Research Network
Réseau canadien de recherche
sur la mammite bovine

MASTITIS MANAGEMENT

questionnaire

25 – 30 minutes
to fill in

For any questions and inquiries please contact:

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This project is fund

Dairy Farmers of C
Canadian Bovine Mastitis Research N
Natural Sciences and Engineering Research Council of C
Atlantic Veterinary C

1. General questions about your farm

1.1 How many female young stock do you have?

Less than 1 year (include new born heifer calves)	[____] head	1.1a [ ]
1 year to calving age	[____] head	1.1b [ ]

1.2 Type of housing for the milking cows, dry cows and bred heifers (√ **all** that apply):

Lactating cows:	Dry cows:	Bred heifers:	
O Tie-stall	O Tie-stall	O Tie-stall	1.2L [ ]
O Free-stall	O Free-stall	O Free-stall	1.2D [ ]
O Other: (please specify)	O Other: (please specify)	O Other: (please specify)	1.2H [ ]
.....	.....	.....	

1.3 Do you have a business goal or mission statement written down on paper?	O Yes O No	1.3 [ ]
1.4 Do you have set goals for udder health performance written down on paper?	O Yes O No	1.4 [ ]

2. Milking procedures

2.1 How many different people have been milking the cows in the last week (include temporary / relief milkers)	_____ people	2.1 [ ]

2.2 What best describes your udder preparation before milking? (√ **all** that apply)

No preparation	O All cows	O Most (≥50%)	O Some (<50%)	O None	2.2a [ ]
Dry wipe only	O All cows	O Most (≥50%)	O Some (<50%)	O None	2.2b [ ]
Predip and dry	O All cows	O Most (≥50%)	O Some (<50%)	O None	2.2c [ ]
Wash	O All cows	O Most (≥50%)	O Some (<50%)	O None	2.2d [ ]

2.3 *If you* <u>wash</u> *the cows' udders,* do you use a disinfectant in the water?	O Yes O No	2.3 [ ]
2.4 Do you <u>dry</u> the cows' udders after washing or dipping?	O Yes O No	2.4 [ ]
2.5 *If you* <u>dry</u> *the cows' udders after washing or dipping,* what do you use?	O Paper towel or newspaper O Cloth or towel O Sponge O Other: (please specify)	2.5 [ ]

2.6 *If you dry the cows' udders after washing or dipping,* how many cows do you dry per towel, cloth or sponge?	_____ cow(s)	2.6 [ ]
2.7 *If you do pre-dip*, which brand do you use?	O Della Pretech® (DeLaval) O Theratec® (WestfaliaSurge) O Ready-Wipe® O Other: (please specify)	2.7 [ ]
2.8 Do you and your milkers wear latex (or similar) gloves during milking?	O Yes O Sometimes O No	2.8 [ ]
2.9 Does your equipment have automated takeoffs?	O Yes O No	2.9 [ ]
2.10 *If not,*do you shut the vacuum off before or after cup removal?	O Before O After	2.10 [ ]
Teat disinfection		
2.11 Do you apply post-milking disinfection to the cows teats (dip or spray) ?	O Yes O No	2.11 [ ]
2.12 *If you do post-milking disinfection,* do you use:	O Dipping cups O Manual sprayer O Automated sprayer	2.12 [ ]
2.13 *If you do post-milking disinfection*, which brand do you use?	O *Same as pre-milking* O Della One® (DeLaval) O Teat-Kote® 10/III (WestfaliaSurge) O Bovi-Kote® (Bou-Matic) O Other: (please specify)	2.13 [ ]
Milking order		
2.14 Do you milk cows with a high (Somatic) Cell Count (SCC) last **and/or** with a separate unit?	O Yes O No	2.14 [ ]
2.15 Do you milk *Staphylococcus aureus* infected cows last **and/or** with a separate unit?	O Yes O No	2.15 [ ]
2.16 Do you milk cows with clinical mastitis last **and/or** with a separate unit?	O Yes O No	2.16 [ ]

2.17 Do your cows have access to fresh feed and water during milking or *immediately* (maximum 15 minutes) after they have been milked?

Access to fresh feed O Yes O No 2.17a [ ]

Access to fresh water O Yes O No 2.17b [ ]

3. Management of clinical cases

3.1 How is clinical mastitis commonly seen or detected on your farm? (please circle the appropriate value)

	4 = very often - - - 1 = rarely	
Abnormal milk	4 - 3 - 2 - 1 (very often / rarely)	3.1a [ ]
Abnormal udder	4 - 3 - 2 - 1 (very often / rarely)	3.1b [ ]
Abnormal kicking during milking	4 - 3 - 2 - 1 (very often / rarely)	3.1c [ ]
Sick cow	4 - 3 - 2 - 1 (very often / rarely)	3.1d [ ]

3.2 Do you collect milk samples of newly diagnosed clinical mastitis cases?

O All cases
O Most cases (≥50%)
O Some cases (<50%)
O No cases 3.2 [ ]

Treatment

3.3 Do you treat all cases of mastitis with antibiotics?

O All cases
O Most cases (≥50%)
O Some cases (<50%)
O No cases 3.3 [ ]

3.4 Which person, media or company was important in the decision of the type or brand of *lactating cow* treatment that you use?

5 = very important - - 1 = not important

Veterinarian	5 - 4 - 3 - 2 - 1 (very important / not important)	3.4a [ ]
Other farmers	5 - 4 - 3 - 2 - 1 (very important / not important)	3.4b [ ]
Local farm supplier	5 - 4 - 3 - 2 - 1 (very important / not important)	3.4c [ ]
Advertisement	5 - 4 - 3 - 2 - 1 (very important / not important)	3.4d [ ]
Other: (please specify)	5 - 4 - 3 - 2 - 1 (very important / not important)	3.4e [ ]

3.5 Do you use specially made products (prepared by yourself or by your veterinarian) as opposed to commercial available products?
O Yes O Sometimes O No — 3.5 [ ]

3.6 *If you use commercial products,* do you use full (longtip) or partial (shorttip) insertion?
O Full O Partial — 3.6 [ ]

3.7 Do you disinfect the teat with an alcohol swab before infusion?
O Yes O Sometimes O No — 3.7 [ ]

3.8 How many treatments do you apply to a cow as a maximum if she does not clear up?
____ treatments — 3.8 [ ]

3.9 Can you indicate how important the fact that she does not clear up is in culling the cow?
very important ... not important
5 - 4 - 3 - 2 - 1 — 3.9 [ ]

3.10 How do you mark or remember that a cow has been treated? (√ **all** that apply)

O The cow's name or ID on a white board or chalk board	3.10a [ ]
O Keep her separated	3.10b [ ]
O Apply (colored) leg bands	3.10c [ ]
O Color mark (leg, back, udder, tail, etc.)	3.10d [ ]
O Other: (please specify)	3.10e [ ]

3.11 Do you vaccinate your cows against mastitis?

O All cows	
O Most of them (≥ 50%)	
O Some (< 50%)	
O None	3.11 [ ]

4. Dry cow management

4.1 What proportion of cows are dry-cow treated at the end of lactation with antibiotics? _____ % — 4.1 [ ]

4.2 *If you use Orbeseal®, what proportion of cows are dry-cow treated with Orbeseal®?* _____ % — 4.2 [ ]

4.3 Which products do you use? (√ **all** that apply)

O Dryclox® — 4.3a [ ]

O Cefadry® — 4.3b [ ]

O Orbeseal® — 4.3c [ ]

O Other: (please specify) ..... — 4.3d [ ]

4.4a Do you disinfect the teat with an alcohol swab before infusion? O Yes O Sometimes O No — 4.4a [ ]

4.4b Do you use full (longtip) or partial (shorttip) insertion? O Full O Partial — 4.4b [ ]

4.5 Do you use a teat dip or spray after infusion? O Yes O Sometimes O No — 4.5 [ ]

Feed and water reduction

4.6 How many days before drying off do you reduce feed energy levels?

O _____ days before dry-off

O No feed or energy reduction — 4.6 [ ]

4.7 How many days before drying off do you reduce water intake?

O _____ days before dry-off

O No water reduction — 4.7 [ ]

5. Cows with high Somatic Cell Counts (SCC)

5.1 Do you have equipment for a California Mastitis Test (CMT) or Rapid Mastitis Test (RMT) on your farm? O Yes O No — 5.1 [ ]

5.2 *If you have the equipment on farm,* how frequently do you use this equipment?

O More than once a month

O Less than once a month, but more than twice a year

O Once a year or less — 5.2 [ ]

5.3 Do you take milk samples from cows with high SCC for bacterial culture? O Yes O Sometimes O No — 5.3 [ ]

5.4 Can you indicate how important permanent high (somatic) cell counts are in the decision to cull cow?

5 - 4 - 3 - 2 - 1
very important not important

5.4 [ ]

5.5 *If you send in milk samples,* can you indicate how important an infection with *Staphylococcus aureus* is in culling cows?

5 - 4 - 3 - 2 - 1
very important not important

5.5 [ ]

6. Milking equipment

6.1 What is the main brand name of your equipment?

O WestfaliaSurge
O DeLaval
O Bou-Matic
O Other: (please specify)
.....

6.1 [ ]

6.2 How often is the functioning of your milking equipment checked and analysed by the *equipment dealer*?

O Twice or more times per year
O Once a year
O Less than once a year
O Never

6.2 [ ]

6.3 How often is the functioning of your milking equipment checked and analysed by *an independent technician*?

O Twice or more times per year
O Once a year
O Less than once a year
O Never

6.3 [ ]

7. Record keeping

7.1 Do you use a computer for keeping records of your cows?

O Yes O No

7.1 [ ]

7.2 If yes, which dairy management program do you use?

O DairyComp305 / Scout
O VAMPP
O DairyChamp
O Other: (please specify)
.....

7.2 [ ]

7.3 In which record system do you keep records of *clinical mastitis cases*? (√ **all** that apply)

O None	7.3a [ ]
O White board, chalk board or similar	7.3b [ ]
O Cow cards	7.3c [ ]
O Breeding wheel	7.3d [ ]
O A 21-day calendar	7.3e [ ]
O Cow diary	7.3f [ ]
O Computer	7.3g [ ]
O Other: (please specify)	7.3h [ ]

7.4 Which data do you record of each clinical mastitis case? (√ **all** that apply)

O Cow name or number	7.4a [ ]
O Which quarter is infected	7.4b [ ]
O Severity	7.4c [ ]
O Date of onset	7.4d [ ]
O Date of last treatment	7.4e [ ]
O Type of treatment	7.4f [ ]
O Number of treatments	7.4g [ ]
O Date of return in bulk tank	7.4h [ ]
O Type of bacteria after culture	7.4i [ ]

8. Cow comfort and hygiene

Please answer the question for lactating cows, dry cows and bred heifers separately

8.1 What material does the stall base consist of (√ **all** that apply)?

Lactating cows	Dry cows	Bred heifers	
O Concrete	O Concrete	O Concrete	
O Mattresses	O Mattresses	O Mattresses	
O Rubber mat	O Rubber mat	O Rubber mat	8.1L [ ]
O Clay	O Clay	O Clay	8.1D [ ]
O Other: (please specify)	O Other: (please specify)	O Other: (please specify)	8.1H [ ]

8.2 What material do you use as bedding (√ **all** that apply)?

Lactating cows	Dry cows	Bred heifers	
O None	O None	O None	
O Sawdust	O Sawdust	O Sawdust	
O Shavings	O Shavings	O Shavings	
O Sand	O Sand	O Sand	8.2L [ ]
O Straw	O Straw	O Straw	8.2D [ ]
O Other: (please specify)	O Other: (please specify)	O Other: (please specify)	8.2H [ ]
.....	.....	.....	

8.3 How often do you clean out the manure in the stalls(√ **all** that apply)?

Lactating cows	Dry cows	Bred heifers	
O Twice a day	O Twice a day	O Twice a day	
O Once a day	O Once a day	O Once a day	8.3L [ ]
O Once every two days	O Once every two days	O Once every two days	8.3D [ ]
O Other: (please specify)	O Other: (please specify)	O Other: (please specify)	8.3H [ ]
.....	.....	.....	

8.4 How often do you change the bedding in the stalls (√ **all** that apply)?

Lactating cows	Dry cows	Bred heifers	
O Once a day	O Once a day	O Once a day	
O Once every two days	O Once every two days	O Once every two days	8.4L [ ]
O Twice a week	O Twice a week	O Twice a week	8.4D [ ]
O Other: (please specify)	O Other: (please specify)	O Other: (please specify)	8.4H [ ]
.....	.....	.....	

8.5 *If you have a free-stall,* how are the alleys cleaned (√ **all** that apply)?

O Manual
O Automated scraper
O Other: (please specify)
.....

8.5 [ ]

8.6 *If you have a free-stall,* how often are the alleys scraped (manual or scraper)?

O _____ times a day

8.6 [ ]

Cow hygiene

8.7 Do you clip or flame udders?
O No
O Clip
O Flame
8.7 [ ]

8.8 *If you do clip or flame,* how often is each cow clipped or flamed?
..... times per year
8.8 [ ]

8.9 Do you clip or dock tails
O No
O Clip
O Dock
8.9 [ ]

8.10 *If you do clip tails,* how often is each cow's tail clipped?
..... times per year
8.10 [ ]

9. Biosecurity

Purchasing animals and animal contact

9.1 How many heifers and cows on your farm are purchased animals?
_____ heifers purchased
9.1H [ ]

_____ cows purchased
9.1C [ ]

9.2 *If you purchase cows,* do you take and test milk samples of cows prior to purchase?
O Always take milk samples
O Usually (≥50%)
O Sometimes (<50%)
O Never
9.2 [ ]

9.3 *If you purchase cows,* do you request information on (Somatic) Cell Counts prior to purchase?
O Always request SCC information
O Usually (≥50%)
O Sometimes (<50%)
O Never
9.3 [ ]

9.4 Do you treat heifers with injectable antibiotics prior to calving as a mastitis prevention measure?
O Yes O Sometimes O No
9.4 [ ]

10. Nutrition

10.1 How often are the cows' rations balanced based on forage analyses?

O Three or more times a year
O Twice a year
O Once a year
O Less than once a year
O Never

10.1 [ ]

10.2 In formulating the cow's rations, can you indicate the role of each of the following persons:

	5 = very important - - 1 = not important	
Independent nutritionist	5 - 4 - 3 - 2 - 1 (very important / not important)	10.2a [ ]
Feed company representative	5 - 4 - 3 - 2 - 1 (very important / not important)	10.2b [ ]
Veterinarian	5 - 4 - 3 - 2 - 1 (very important / not important)	10.2c [ ]
DHI representative or equivalent (WDHIA, MMB, ODHI, PATLQ, ADLIC)	5 - 4 - 3 - 2 - 1 (very important / not important)	10.2d [ ]
Other: (please specify)	5 - 4 - 3 - 2 - 1 (very important / not important)	10.2e [ ]

11. Mastitis plan review

11.1 Who is important in reviewing your mastitis data and /or plan with you?

	5 = very important - - - 1 = not important	
Veterinarian	5 - 4 - 3 - 2 - 1 (very important / not important)	11.1a [ ]
DHI representative or equivalent (WDHIA, MMB, ODHI, PATLQ, ADLIC)	5 - 4 - 3 - 2 - 1 (very important / not important)	11.1b [ ]
Nutritionist	5 - 4 - 3 - 2 - 1 (very important / not important)	11.1c [ ]
Milking equipment representative	5 - 4 - 3 - 2 - 1 (very important / not important)	11.1d [ ]
Neighbor	5 - 4 - 3 - 2 - 1 (very important / not important)	11.1e [ ]
Family member(s)	5 - 4 - 3 - 2 - 1 (very important / not important)	11.1f [ ]
Other: (please specify)	5 - 4 - 3 - 2 - 1 (very important / not important)	11.1g [ ]

11.2 How often do you sit down and review your mastitis data?

O Once a week
O Twice a month
O Once a month
O Twice a year
O Only when my bulk tank somatic cell counts (BMSCC) exceeds 200,000 cells/ml
O Only when my bulk tank somatic cell counts (BMSCC) exceeds 400,000 cells/ml
O Other: (please specify)
.....

11.2 [ ]

Please write your remarks and comments here:

Thank you for your time!

APPENDIX 4

Canadian Bovine Mastitis
Research Network
Réseau canadien de recherche
sur la mammite bovine

RÉGIE DE LA MAMMITE

questionnaire

25 – 30 minutes
à compléter

Pour toutes demandes ou questions, svp contacter:

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Le Réseau canadien de recherche sur la mammite bovine

Conseil de recherches en sciences naturelles et en génie du Canada

Collège Vétérinaire de l'Atlantique

1. Questions générales concernant votre ferme

1.1 Combien de jeunes femelles possédez-vous?

Agées de moins d'un an (incluant les génisses nouvelles-nées) [____] individus 1.1a[ ]

Agées d'un an jusqu'à l'âge au vêlage [____] individus 1.1b[ ]

1.2 Type de logement pour les vaches en lactation, les vaches taries et les taures saillies (√ **tout** ce qui s'applique):

Vaches en lactation:	Vaches taries:	Taures saillies:	
❑ Étable attachée	❑ Étable attachée	❑ Étable attachée	1.2L[ ]
❑ Stabulation libre	❑ Stabulation libre	❑ Stabulation libre	1.2D[ ]
❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	1.2H[ ]
.....	.....	.....	

1.3 Avez-vous des buts définis et une mission d'entreprise écrite sur papier? ❑ Oui ❑ Non 1.3[ ]

1.4 Avez-vous des buts définis concernant la performance en santé du pis et qui sont écrits sur papier? ❑ Oui ❑ Non 1.4[ ]

2. Méthodes de traite

2.1 Combien de personnes ont effectué la traite des vaches au cours de la dernière semaine (incluant les employés temporaires) _____ personnes 2.1[ ]

2.2 Qu'est-ce qui décrit le mieux vos étapes de préparation du pis avant la traite? (√ **tout** ce qui s'applique)

Aucune préparation	❑ Toutes les vaches	❑ La plupart (≥50%)	❑ Quelques-unes (<50%)	❑ Aucune	2.2a[ ]
Essuyage à sec seulement	❑ Toutes les vaches	❑ La plupart (≥50%)	❑ Quelques-unes (<50%)	❑ Aucune	2.2b[ ]
Prétrempage et séchage	❑ Toutes les vaches	❑ La plupart (≥50%)	❑ Quelques-unes (<50%)	❑ Aucune	2.2c[ ]
Lavage	❑ Toutes les vaches	❑ La plupart (≥50%)	❑ Quelques-unes (<50%)	❑ Aucune	2.2d[ ]

2.3 *Si vous lavez le pis des vaches,* utilisez-vous un désinfectant dans l'eau? ❑ Oui ❑ Non 2.3[ ]

2.4 Séchez-vous le pis des vaches après le lavage ou le prétrempage? ❑ Oui ❑ Non 2.4[ ]

2.5 *Si vous séchez le pis des vaches après le lavage ou le prétrempage, quel moyen de séchage utilisez-vous?*

❑ Serviette de papier ou papier journal
❑ Chiffon ou lavette
❑ Éponge
❑ Autre: (svp spécifier)
.....

2.5[ ]

2.6 *Si vous séchez le pis des vaches après le lavage ou le prétrempage,* combien de vaches sont-elles séchées avec la même serviette, lavette ou éponge? _____ vache(s) — 2.6[ ]

2.7 *Si vous effectuez le prétrempage*, quelle marque de produit utilisez-vous?

- ❑ Della Pretech® (DeLaval)
- ❑ Theratec® (WestfaliaSurge)
- ❑ Ready-Wipe®
- ❑ Autre: (svp spécifier) .....

2.7[ ]

2.8 Est-ce que vos trayeurs et vous-même portez des gants de latex (ou similaire) durant la traite? ❑ Oui ❑ Parfois ❑ Non — 2.8[ ]

2.9 Votre équipement de traite est-il muni du système de retrait automatique? ❑ Oui ❑ Non — 2.9[ ]

2.10 *Si non,* coupez-vous le vide avant ou après le retrait des manchons trayeurs? ❑ Avant ❑ Après — 2.10[ ]

Désinfection des trayons

2.11 Effectuez-vous la désinfection post-traite des trayons (trempage ou pulvérisation)? ❑ Oui ❑ Non — 2.11[ ]

2.12 *Si vous effectuez une désinfection post-traite,* utilisez-vous:

- ❑ Le trempage
- ❑ La pulvérisation manuelle
- ❑ La pulvérisation automatique

2.12[ ]

2.13 *Si vous effectuez une désinfection post-traite,* quelle marque de produit utilisez-vous?

- ❑ *La même que pour le prétrempage*
- ❑Della One® (DeLaval)
- ❑ Teat-Kote® 10/III (WestfaliaSurge)
- ❑ Bovi-Kote® (Bou-Matic)
- ❑ Autre: (svp spécifier) .....

2.13[ ]

Routine de la traite

2.14 Trayez-vous les vaches ayant un comptage de cellules somatiques (CCS) élevé en dernier **et/ou** avec une unité séparée? ❑ Oui ❑ Non — 2.14[ ]

2.15 Trayez-vous les vaches infectées à *Staphylococcus aureus* en dernier **et/ou** avec une unité séparée? ❑ Oui ❑ Non — 2.15[ ]

2.16 Trayez-vous les vaches ayant une mammite clinique en dernier **et/ou** avec une unité séparée? ❑ Oui ❑ Non — 2.16[ ]

2.17 Vos vaches ont-elles accès à des aliments frais et de l'eau fraîche durant la traite ou *immédiatement* (maximum 15 minutes) après qu'elles aient été traites?

Accès à des aliments frais ❑ Oui ❑ Non 2.17a[ ]

Accès à de l'eau fraîche ❑ Oui ❑ Non 2.17b[ ]

3. Régie des cas de mammite clinique

3.1 De quelle(s) façon(s) les cas de mammite clinique sont-ils communément observés ou détectés sur votre ferme? (svp encercler la valeur appropriée)

4 = très souvent - - - 1 = rarement

Lait anormal 4 - 3 - 2 - 1 (très souvent / rarement) 3.1a[ ]

Pis anormal 4 - 3 - 2 - 1 (très souvent / rarement) 3.1b[ ]

Agitation anormale durant la traite (coups de patte) 4 - 3 - 2 - 1 (très souvent / rarement) 3.1c[ ]

Vache malade 4 - 3 - 2 - 1 (très souvent / rarement) 3.1d[ ]

3.2 Récoltez-vous des échantillons de lait chez les cas de mammite clinique nouvellement diagnostiqués?

❑ Dans tous les cas
❑ Dans la plupart des cas (≥50%)
❑ Dans quelques cas (<50%)
❑ En aucun cas 3.2[ ]

Traitement

3.3 Traitez-vous tous les cas de mammite avec des antibiotiques?

❑ Dans tous les cas
❑ Dans la plupart des cas (≥50%)
❑ Dans quelques cas (<50%)
❑ En aucun cas 3.3[ ]

3.4 Quelle intervenant, quel média ou quelle compagnie a eu une influence importante sur votre décision concernant le type ou la marque du traitement pour *vache en lactation* que vous utilisez?

5 = très important - - 1 = pas important

Vétérinaire	5 - 4 - 3 - 2 - 1 (très important / pas important)	3.4a[ ]
Autres producteurs laitiers	5 - 4 - 3 - 2 - 1 (très important / pas important)	3.4b[ ]
Fournisseur local	5 - 4 - 3 - 2 - 1 (très important / pas important)	3.4c[ ]
Publicité	5 - 4 - 3 - 2 - 1 (très important / pas important)	3.4d[ ]
Autre: (svp spécifier)	5 - 4 - 3 - 2 - 1 (très important / pas important)	3.4e[ ]

3.5 Utilisez-vous des produits spécialement préparés (par vous-même ou par votre vétérinaire) au lieu de produits disponibles commercialement? ❑ Oui ❑ Parfois ❑ Non — 3.5[ ]

3.6 *Si vous utilisez des produits commerciaux,* utilisez-vous l'insertion complète (long bout) ou partielle (court bout)? ❑ Complète ❑ Partielle — 3.6[ ]

3.7 Désinfectez-vous le trayon avec un tampon d'alcool avant l'infusion? ❑ Oui ❑ Parfois ❑ Non — 3.7[ ]

3.8 Quel nombre maximum de traitements administrez-vous à une vache si la situation ne s'améliore pas? ____ traitements — 3.8[ ]

3.9 Indiquez l'importance d'une mammite qui ne s'améliore pas dans votre décision de réformer une vache? très important 5 - 4 - 3 - 2 - 1 pas important — 3.9[ ]

3.10 De quelle façon vous souvenez-vous ou notez-vous qu'une vache a reçu un traitement? (√ **tout** ce qui s'applique)

- ❑ Le nom ou le numéro de la vache sur un tableau blanc ou noir à la craie? — 3.10a[ ]
- ❑ Garder la vache séparée — 3.10b[ ]
- ❑ Apposer un bracelet coloré sur une patte — 3.10c[ ]
- ❑ Marquer la vache d'une couleur (jambe, dos, pis, queue, etc.) — 3.10d[ ]
- ❑ Autre: (svp spécifier) ..... — 3.10e[ ]

3.11 Vaccinez-vous vos vaches contre la mammite?

- ❑ Toutes les vaches
- ❑ La plupart des vaches (≥ 50%)
- ❑ Quelques vaches (< 50%)
- ❑ Aucune — 3.11[ ]

4. Traitement au tarissement

4.1 Quelle proportion de vos vaches reçoivent un traitement au tarissement avec des antibiotiques à la fin de la lactation? _____ % 4.1[ ]

4.2 *Si vous utilisez Orbeseal®* dans quelle proportion de vos vaches reçoivent un traitement au tarissement avec *Orbeseal®*? _____ % 4.2[ ]

4.3 Quels produits utilisez-vous en période de tarissement? (√ **tout** ce qui s'applique)

- ❑ Dryclox® 4.3a[ ]
- ❑ Cefadry® 4.3b[ ]
- ❑ Orbeseal® 4.3c[ ]
- ❑ Autre: (svp spécifier) 4.3d[ ]

.....

4.4a Désinfectez-vous le trayon avec un tampon d'alcool avant l'infusion? ❑ Oui ❑ Parfois ❑ Non 4.4a[ ]

4.4b Utilisez-vous l'insertion complète (long bout) ou partielle (court bout)? ❑ Complète ❑ Partielle 4.4b[ ]

4.5 Après l'infusion, effectuez-vous un bain de trayon (trempage ou pulvérisation)? ❑ Oui ❑ Parfois ❑ Non 4.5[ ]

Diminution des aliments et de l'eau

4.6 Combien de jours précédant le tarissement réduisez-vous les niveaux d'énergie alimentaire?

- ❑ _____ jours avant le tarissement
- ❑ Aucune diminution dans la quantité d'aliments ou du niveau d'énergie 4.6[ ]

4.7 Combien de jours précédant le tarissement réduisez-vous la consommation en eau?

- ❑ _____ jours avant le tarissement
- ❑ Aucune diminution de l'eau 4.7[ ]

5. Vaches avec haut comptage de celiules somatiques (CCS)

5.1 Avez-vous l'équipement nécessaire pour effectuer un « California Mastitis Test » (CMT) ou un « Rapid Mastitis Test » (RMT) à la ferme? ❑ Oui ❑ Non 5.1[ ]

5.2 *Si vous avez l'équipement à la ferme,* à quelle fréquence l'utilisez-vous?

- ❑ Plus d'une fois par mois
- ❑ Moins d'une fois par mois, mais plus de deux fois par année
- ❑ Une fois par année ou moins 5.2[ ]

5.3 Récoltez-vous des échantillons de lait des vaches avec un CCS élevé pour effectuer des cultures bactériologiques? ❑ Oui ❑ Parfois ❑ Non 5.3[ ]

5.4 Indiquez l'importance des CCS élevés permanents dans votre décision de réformer une vache? 5 - 4 - 3 - 2 - 1 (très important — pas important) 5.4[ ]

5.5 *Si vous faites analyser des échantillons de lait,* indiquez l'importance d'une infection avec *Staphylococcus aureus* dans votre décision de réformer une vache?

5 - 4 - 3 - 2 - 1

très important — pas important

5.5[ ]

6. Équipement de traite

6.1 Quelle est la marque principale de votre équipement de traite?

- ❑ WestfaliaSurge
- ❑ DeLaval
- ❑ Bou-Matic
- ❑ Autre: (svp spécifier)

.....

6.1[ ]

6.2 À quelle fréquence faites-vous vérifier et analyser le fonctionnement de votre équipement de traite par le marchand d'équipements?

- ❑ Deux fois ou plus par année
- ❑ Une fois par année
- ❑ Moins d'une fois par année
- ❑ Jamais

6.2[ ]

6.3 À quelle fréquence faites-vous vérifier et analyser le fonctionnement de votre équipement de traite par *un technicien indépendant?*

- ❑ Deux fois ou plus par année
- ❑ Une fois par année
- ❑ Moins d'une fois par année
- ❑ Jamais

6.3[ ]

7. Tenue de dossier

7.1 Utilisez-vous un ordinateur pour la tenue de dossiers de vos vaches? ❑ Oui ❑ Non

7.1[ ]

7.2 Si oui, quel logiciel de régie de production laitière utilisez-vous?

- ❑ DairyComp305 / Scout
- ❑ VAMPP
- ❑ DairyChamp
- ❑ DSA (vétérinaire)
- ❑ Agri-Lacta
- ❑ Autre: (svp spécifier)

.....

7.2[ ]

7.3 Quel système utilisez-vous pour la tenue de dossier des *cas de mammite clinique?* (√ **tout** ce qui s'applique)

- ❑ Aucun — 7.3a[ ]
- ❑ Tableau blanc ou noir à craie ou similaire — 7.3b[ ]

	❑ Cartes de vaches	7.3c[ ]
	❑ Tableau de régie	7.3d[ ]
	❑ Calendrier de 21 jours	7.3e[ ]
	❑ Carnet de régie	7.3f[ ]
	❑ Ordinateur	7.3g[ ]
	❑ Autre: (svp spécifier)	7.3h[ ]
7.4 Quelles données consignez-vous dans les cas de mammite clinique? (√ **tout** ce qui s'applique)		
	❑ Nom ou numéro de la vache	7.4a[ ]
	❑ Quartier infecté	7.4b[ ]
	❑ Sévérité	7.4c[ ]
	❑ Date de début des signes	7.4d[ ]
	❑ Date du dernier traitement	7.4e[ ]
	❑ Type de traitement	7.4f[ ]
	❑ Nombre de traitements	7.4g[ ]
	❑ Date de retour dans le réservoir de lait	7.4h[ ]
	❑ Types de bactéries observées à la culture	7.4i[ ]

8. Confort et hygiène de la vache

SVP répondre à la question séparément pour les vaches en lactation, les vaches taries et les taures saillies.

8.1 Quel matériel est utilisé comme base de la stalle (√ **tout** ce qui s'applique)?

Vaches en lactation	Vaches taries	Taures saillies	
❑ Ciment	❑ Ciment	❑ Ciment	
❑ Matelas	❑ Matelas	❑ Matelas	
❑ Matelas en caoutchouc	❑ Matelas en caoutchouc	❑ Matelas en caoutchouc	8.1L[ ]
❑ Argile	❑ Argile	❑ Argile	8.1D[ ]
❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	8.1H[ ]

8.2 Quel type de litière utilisez-vous (√ **tout** ce qui s'applique)?

Vaches en lactation	Vaches taries	Taures saillies
❑ Aucune	❑ Aucune	❑ Aucune

❑ Sciure de bois	❑ Sciure de bois	❑ Sciure de bois	
❑ Copeaux de bois	❑ Copeaux de bois	❑ Copeaux de bois	
❑ Sable	❑ Sable	❑ Sable	8.2L[ ]
❑ Paille	❑ Paille	❑ Paille	8.2D[ ]
❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	8.2H[ ]
.....	.....	.....	

8.3 À quelle fréquence nettoyez-vous le fumier dans les stalles (√ **tout** ce qui s'applique)?

Vaches en lactation	Vaches taries	Taures saillies	
❑ Deux fois par jour	❑ Deux fois par jour	❑ Deux fois par jour	
❑ Une fois par jour	❑ Une fois par jour	❑ Une fois par jour	8.3L[ ]
❑ Une fois par deux jours	❑ Une fois par deux jours	❑ Une fois par deux jours	8.3D[ ]
❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	8.3H[ ]
.....	.....	.....	

8.4 À quelle fréquence changez-vous la litière dans les stalles (√ **tout** ce qui s'applique)?

Vaches en lactation	Vaches taries	Taures saillies	
❑ Une fois par jour	❑ Une fois par jour	❑ Une fois par jour	
❑ Une fois par deux jours	❑ Une fois par deux jours	❑ Une fois par deux jours	8.4L[ ]
❑ Deux fois par semaine	❑ Deux fois par semaine	❑ Deux fois par semaine	8.4D[ ]
❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	❑ Autre: (svp spécifier)	8.4H[ ]
.....	.....	.....	

8.5 *Si vous avez une stabulation libre,* de quelle(s) façon(s) nettoyez-vous les allées?

(√ **tout** ce qui s'applique)

❑ Nettoyage manuel

❑ Raclette automatique

❑ Autre: (svp spécifier)

.....

8.5[ ]

8.6 *Si vous avez une stabulation libre,* à quelle fréquence les allées sont-elles nettoyées (manuel ou raclette)?

❑ _____ fois par jour

8.6[ ]

Hygiène de la vache

8.7 Coupez-vous le poil du pis (à la torche ou avec une tondeuse)?

❑ Non

❑ Tondeuse

	❑ Torche	8.7[ ]
8.8 *Si vous coupez le poil du pis,* à quelle fréquence le faites-vous?	..... fois par année	8.8[ ]
8.9 Rasez-vous ou coupez vous les queues?	❑ Non	
	❑ Raser	
	❑ Couper	8.9[ ]
8.10 *Si vous rasez les queues,* à quelle fréquence le faites-vous?	..... fois par année	8.10[ ]

9. Biosécurité

Achat d'animaux et contact entre animaux

9.1 Combien de vos taures et de vos vaches ont-elles été achetées?	_____ taures achetées	9.1H[ ]
	_____ vaches achetées	9.1C[ ]
9.2 *Si vous achetez des vaches,* récoltez-vous et analysez-vous des échantillons de leur lait avant l'achat?	❑ Je prends toujours des échantillons de lait	
	❑ Habituellement (≥50%)	
	❑ Parfois (<50%)	
	❑ Jamais	9.2[ ]
9.3 Si vous achetez des vaches, demandez-vous des informations concernant le niveau du comptage des cellules somatiques (CSS) avant l'achat?	❑ Je demande toujours l'information sur le CSS	
	❑ Habituellement (≥50%)	
	❑ Parfois (<50%)	
	❑ Jamais	9.3[ ]
9.4 Traitez-vous les taures avec des antibiotiques injectables avant le vêlage comme mesure de prévention de la mammite?	❑ Oui ❑ Parfois ❑ Non	9.4[ ]

10. Nutrition

10.1 À quelle fréquence balancez-vous les rations des vaches en vous basant sur les analyses de fourrages?	❑ Trois fois ou plus par année	
	❑ Deux fois par année	

❑ Une fois par année
❑ Moins d'une fois par année
❑ Jamais — 10.1[ ]

10.2 Pouvez-vous indiquer le rôle des intervenants suivants dans la formulation des rations de vos vaches:

	5 = très important - - 1 = pas important	
Nutritionniste indépendant	5 - 4 - 3 - 2 - 1 (très important / pas important)	10.2a[ ]
Représentant de compagnie d'alimentation	5 - 4 - 3 - 2 - 1 (très important / pas important)	10.2b[ ]
Vétérinaire	5 - 4 - 3 - 2 - 1 (très important / pas important)	10.2c[ ]
Représentant du centre de contrôle laitier ou équivalent (PATLQ, WDHIA, MMB, ODHI, ADLIC)	5 - 4 - 3 - 2 - 1 (très important / pas important)	10.2d[ ]
Autre: (svp spécifier)	5 - 4 - 3 - 2 - 1 (très important / pas important)	10.2e[ ]

11. Révision du plan de régie de la mammite

11.1 Qui est l'intervenant important pour réviser vos données ou votre plan de régie de la mammite?

	5 = très important - - 1 = pas important	
Vétérinaire	5 - 4 - 3 - 2 - 1 (très important / pas important)	11.1a[ ]
Représentant du centre de contrôle laitier ou équivalent (PATLQ, WDHIA, MMB, ODHI, ADLIC)	5 - 4 - 3 - 2 - 1 (très important / pas important)	11.1b[ ]
Nutritionniste	5 - 4 - 3 - 2 - 1 (très important / pas important)	11.1c[ ]
Représentant d'équipement de traite	5 - 4 - 3 - 2 - 1 (très important / pas important)	11.1d[ ]
Voisin	5 - 4 - 3 - 2 - 1 (très important / pas important)	11.1e[ ]
Membre(s) de la famille	5 - 4 - 3 - 2 - 1 (très important / pas important)	11.1f[ ]
Autre: (svp spécifier)	5 - 4 - 3 - 2 - 1 (très important / pas important)	11.1g[ ]

11.2 À quelle fréquence prenez-vous le temps de réviser vos données concernant la mammite?

❑ Une fois par semaine
❑ Deux fois par mois
❑ Une fois par mois
❑ Deux fois par année

❑ Seulement lorsque le CSS de mon réservoir de lait excède 200,000 cellules/ml

❑ Seulement lorsque le CSS de mon réservoir de lait excède 400,000 cellules/ml

❑ Autre: (svp spécifier)

.....

11.2[ ]

Svp écrire vos remarques et commentaires ici:

Merci de votre collaboration!